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(54) Title: PRODUCTION OF FIBRINOGEN IN TRANSGENIC ANIMALS			
(57) Abstract <p>Materials and methods for producing fibrinogen in transgenic non-human mammals are disclosed. DNA segments encoding $\text{A}\alpha$, $\text{B}\beta$ and γ chains of fibrinogen are introduced into the germ line of a non-human mammal, and the mammal or its female progeny produces milk containing fibrinogen expressed from the introduced DNA segments. Non-human mammalian embryos and transgenic non-human mammals carrying DNA segments encoding heterologous fibrinogen polypeptide chains are also disclosed.</p>			

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Description

5 PRODUCTION OF FIBRINOGEN IN TRANSGENIC ANIMALS

Background of the Invention

10 The final step in the blood coagulation cascade is the thrombin-catalyzed conversion of the soluble plasma protein fibrinogen to insoluble fibrin. Thrombin cleaves a small peptide (fibrinopeptide A) from one of the three component chains (the A α -chain) of fibrinogen. Fibrin monomers subsequently polymerize and are cross-linked by activated factor XIII to form a stable clot.

15 Fibrinogen is a key component of biological tissue glues (see, e.g., U.S. Patents Nos. 4,377,572 and 4,442,655), which mimic the formation of natural blood clots to promote hemostasis and repair damaged tissue. Tissue glues provide an adjunct or alternative to sutures, 20 staples and other mechanical means for wound closure. However, the principal ingredients of these products (fibrinogen, factor XIII and thrombin) are prepared from pooled human plasma by cryoprecipitation (e.g. U.S. Patents No. 4,377,572; 4,362,567; 4,909,251) or ethanol 25 precipitation (e.g. U.S. Patent No. 4,442,655) or from single donor plasma (e.g. U.S. Patent No. 4,627,879; Spotnitz et al., Am. Surg. 55: 166-168, 1989). The resultant fibrinogen/factor XIII preparation is mixed with bovine thrombin immediately before use to convert the 30 fibrinogen to fibrin and activate the factor XIII, thus initiating coagulation of the adhesive.

Commercially available adhesives are of pooled plasma origin. Because blood-derived products have been associated with the transmission of human immunodeficiency 35 virus (HIV), hepatitis virus and other etiologic agents, the acceptance and availability of such adhesives is

limited. At present they are not approved for use in the United States.

While the use of autologous plasma reduces the risk of disease transmission, autologous adhesives can 5 only be used in elective surgery when the patient is able to donate the necessary blood in advance.

As noted above, fibrinogen consists of three polypeptide chains, each of which is present in two copies in the assembled molecule. These chains, designated the 10 $\text{A}\alpha$, $\text{B}\beta$ and γ -chains, are coordinately expressed, assembled and secreted by the liver. While it might be expected that recombinant DNA technology could provide an alternative to the isolation of fibrinogen from plasma, this goal has proven to be elusive. The three fibrinogen 15 chains have been individually expressed in *E. coli* (Lord, DNA 4: 33-38, 1985; Bolyard and Lord, Gene 66: 183-192, 1988; Bolyard and Lord, Blood 73: 1202-1206), but functional fibrinogen has not been produced in a prokaryotic system. Expression of biologically competent 20 fibrinogen in yeast has not been reported. Cultured transfected mammalian cells have been used to express biologically active fibrinogen (Farrell et al., Blood 74: 55a, 1989; Hartwig and Danishefsky, J. Biol. Chem. 266: 6578-6585, 1991; Farrell et al., Biochemistry 30: 9414- 25 9420, 1991), but expression levels have been so low that production of recombinant fibrinogen in commercial quantities is not feasible. Experimental evidence suggests that lower transcription rates in cultured cells as compared to liver may be a factor in the low expression 30 rates achieved to date, but increasing the amount of fibrinogen chain mRNA in transfected BHK cells did not produce corresponding increases in fibrinogen protein secretion (Prunkard and Foster, XIV Congress of the International Society on Thrombosis and Haemostasis, 35 1993). These latter results suggest that proper assembly and processing of fibrinogen involves tissue-specific mechanisms not present in common laboratory cell lines.

There remains a need in the art for methods of producing large quantities of high quality fibrinogen for use in tissue adhesives and other applications. There is a further need for fibrinogen that is free of blood-borne 5 pathogens. The present invention fulfills these needs and provides other, related advantages.

Summary of the Invention

It is an object of the present invention to 10 provide commercially useful quantities of recombinant fibrinogen, particularly recombinant human fibrinogen. It is a further object of the invention to provide materials and methods for expressing fibrinogen in the mammary tissue of transgenic animals, particularly livestock 15 animals such as cattle, sheep, pigs and goats.

Within one aspect, the present invention provides a method for producing fibrinogen comprising (a) providing a first DNA segment encoding a secretion signal operably linked to a fibrinogen $A\alpha$ chain, a second DNA 20 segment encoding a secretion signal operably linked to a fibrinogen $B\beta$ chain, and a third DNA segment encoding a secretion signal operably linked to a fibrinogen γ chain, wherein each of the first, second and third segments is operably linked to additional DNA segments required for 25 its expression in the mammary gland of a host female mammal; (b) introducing the DNA segments into a fertilized egg of a non-human mammalian species; (c) inserting the egg into an oviduct or uterus of a female of the species to obtain offspring carrying the DNA constructs; (d) 30 breeding the offspring to produce female progeny that express the first, second and third DNA segments and produce milk containing biocompetent fibrinogen encoded by the segments; (e) collecting milk from the female progeny; and (f) recovering the fibrinogen from the milk. Within 35 one embodiment, the egg containing the introduced segments is cultured for a period of time prior to insertion.

Within another aspect, the invention provides a method of producing fibrinogen comprising the steps of (a) incorporating a first DNA segment encoding a secretion signal operably linked to an $A\alpha$ chain of fibrinogen into a 5 β -lactoglobulin gene to produce a first gene fusion; (b) incorporating a second DNA segment encoding a secretion signal operably linked to a $B\beta$ chain of fibrinogen into a β -lactoglobulin gene to produce a second gene fusion; (c) incorporating a third DNA segment encoding a secretion 10 signal operably linked to a γ chain of fibrinogen into a β -lactoglobulin gene to produce a third gene fusion; (d) introducing the first, second and third gene fusions into the germ line of a non-human mammal so that the DNA segments are expressed in a mammary gland of the mammal or 15 its female progeny and biocompetent fibrinogen is secreted into milk of the mammal or its female progeny; (e) obtaining milk from the mammal or its female progeny; and (f) recovering the fibrinogen from the milk. Within preferred embodiments, the mammal is a sheep, pig, goat or 20 bovine.

Within another aspect, the invention provides a method for producing fibrinogen comprising the steps of (a) providing a transgenic female non-human mammal carrying in its germline heterologous DNA segments 25 encoding $A\alpha$, $B\beta$ and γ chains of fibrinogen, wherein the DNA segments are expressed in a mammary gland of the mammal and fibrinogen encoded by the DNA segments is secreted into milk of the mammal; (b) collecting milk from the mammal; and (c) recovering the fibrinogen from the milk.

30 Within another aspect, the invention provides a non-human mammalian embryo containing in its nucleus heterologous DNA segments encoding $A\alpha$, $B\beta$ and γ chains of fibrinogen. Within a related aspect, the invention provides a transgenic non-human female mammal that 35 produces recoverable amounts of human fibrinogen in its milk.

Within another aspect, the invention provides a method for producing a transgenic offspring of a mammal comprising the steps of (a) providing a first DNA segment encoding a fibrinogen $\text{A}\alpha$ chain, a second DNA segment 5 encoding a fibrinogen $\text{B}\beta$ chain, and a third DNA segment encoding a fibrinogen γ chain, wherein each of said first, second and third segments is operably linked to additional DNA segments required for its expression in a mammary gland of a host female mammal and secretion into milk of 10 the host female mammal; (b) introducing the DNA segments into a fertilized egg of a mammal of a non-human species; (c) inserting the egg into an oviduct or uterus of a female of the non-human species to obtain an offspring carrying the first, second and third DNA segments. In a 15 related aspect, the invention provides non-human mammals produced according to this process.

Within an additional aspect, the invention provides a non-human mammal carrying its germline DNA segments encoding heterologous $\text{A}\alpha$, $\text{B}\beta$ and γ chains of 20 fibrinogen, wherein female progeny of the mammal express the DNA segments in a mammary gland to produce biocompetent fibrinogen.

These and other aspects of the invention will become evident to the skilled practitioner upon reference 25 to the following detailed description and the attached drawings.

Brief Description of the Drawings

Figure 1 illustrates the subcloning of a human fibrinogen A α chain DNA sequence.

Figure 2 is a partial restriction map of the 5 vector Zem228. Symbols used are MT-1p, mouse metallothionein promoter; SV40t, SV40 terminator; and SV40p, SV40 promoter.

Figure 3 illustrates the subcloning of a human fibrinogen B β chain DNA sequence.

10 Figure 4 illustrates the subcloning of a human fibrinogen γ chain DNA sequence.

Figure 5 is a partial restriction map of the vector Zem219b. Symbols used are MT-1p, mouse metallothionein promoter; hGHt, human growth hormone 15 terminator; SV40p, SV40 promoter; DHFR, dihydrofolate reductase gene; and SV40t, SV40 terminator.

Detailed Description of the Invention

Prior to setting forth the invention in detail, 20 it will be helpful to define certain terms used herein:

As used herein, the term "biocompetent fibrinogen" is used to denote fibrinogen that polymerizes when treated with thrombin to form insoluble fibrin.

The term "egg" is used to denote an unfertilized 25 ovum, a fertilized ovum prior to fusion of the pronuclei or an early stage embryo (fertilized ovum with fused pronuclei).

A "female mammal that produces milk containing biocompetent fibrinogen" is one that, following pregnancy 30 and delivery, produces, during the lactation period, milk containing recoverable amounts of biocompetent fibrinogen. Those skilled in the art will recognize that such animals will produce milk, and therefore the fibrinogen, discontinuously.

35 The term "progeny" is used in its usual sense to include children and descendants.

The term "heterologous" is used to denote genetic material originating from a different species than that into which it has been introduced, or a protein produced from such genetic material.

5 Within the present invention, transgenic animal technology is employed to produce fibrinogen within the mammary glands of a host female mammal. Expression in the mammary gland and subsequent secretion of the protein of interest into the milk overcomes many difficulties

10 encountered in isolating proteins from other sources. Milk is readily collected, available in large quantities, and well characterized biochemically. Furthermore, the major milk proteins are present in milk at high concentrations (from about 1 to 15 g/l).

15 From a commercial point of view, it is clearly preferable to use as the host a species that has a large milk yield. While smaller animals such as mice and rats can be used (and are preferred at the proof-of-concept stage), within the present invention it is preferred to

20 use livestock mammals including, but not limited to, pigs, goats, sheep and cattle. Sheep are particularly preferred due to such factors as the previous history of transgenesis in this species, milk yield, cost and the ready availability of equipment for collecting sheep milk.

25 See WO 88/00239 for a comparison of factors influencing the choice of host species. It is generally desirable to select a breed of host animal that has been bred for dairy use, such as East Friesland sheep, or to introduce dairy stock by breeding of the transgenic line at a later date.

30 In any event, animals of known, good health status should be used.

Fibrinogen produced according to the present invention may be human fibrinogen or fibrinogen of a non-human animal. For medical uses, it is preferred to employ

35 proteins native to the patient. The present invention thus provides fibrinogen for use in both human and veterinary medicine. Cloned DNA molecules encoding the

component chains of human fibrinogen are disclosed by Rixon et al. (Biochem. 22: 3237, 1983), Chung et al. (Biochem. 22: 3244, 1983), Chung et al. (Biochem. 22: 3250, 1983), Chung et al. (Adv. Exp. Med. Biol. 281: 39-5 48, 1990) and Chung et al. (Ann. NY Acad. Sci. 408: 449-456, 1983). Bovine fibrinogen clones are disclosed by Brown et al. (Nuc. Acids Res. 17: 6397, 1989) and Chung et al. (Proc. Natl. Acad. Sci. USA 78: 1466-1470, 1981). Other mammalian fibrinogen clones are disclosed by 10 Murakawa et al. (Thromb. Haemost. 69: 351-360, 1993). Representative sequences of human A α , B β and γ chain genes are shown in SEQ ID NOS: 1, 3 and 5, respectively. Those skilled in the art will recognize that allelic variants of these sequences will exist; that additional variants can 15 be generated by amino acid substitution, deletion, or insertion; and that such variants are useful within the present invention. In general, it is preferred that any engineered variants comprise only a limited number of amino acid substitutions, deletions, or insertions, and 20 that any substitutions are conservative. Thus, it is preferred to produce fibrinogen chain polypeptides that are at least 90%, preferably at least 95%, and more preferably 99% or more identical in sequence to the corresponding native chains. The term " γ chain" is meant 25 to include the alternatively spliced γ' chain of fibrinogen (Chung et al., Biochem. 23: 4232-4236, 1984). A human γ' chain amino acid sequence is shown in SEQ ID NO: 6. The shorter γ chain is produced by alternative splicing at nucleotides 9511 and 10054 of SEQ ID NO: 5, 30 resulting in translation terminating after nucleotide 10065 of SEQ ID NO: 5. .

To obtain expression in the mammary gland, a transcription promoter from a milk protein gene is used. Milk protein genes include those genes encoding caseins, 35 beta-lactoglobulin (BLG), α -lactalbumin, and whey acidic protein. The beta-lactoglobulin promoter is preferred. In the case of the ovine beta-lactoglobulin gene, a region

of at least the proximal 406 bp of 5' flanking sequence of the ovine BLG gene (contained within nucleotides 3844 to 4257 of SEQ ID NO:7) will generally be used. Larger portions of the 5' flanking sequence, up to about 5 kbp, 5 are preferred. A larger DNA segment encompassing the 5' flanking promoter region and the region encoding the 5' non-coding portion of the beta-lactoglobulin gene (contained within nucleotides 1 to 4257 of SEQ ID NO:7) is particularly preferred. See Whitelaw et al., Biochem J. 10 286: 31-39, 1992. Similar fragments of promoter DNA from other species are also suitable.

Other regions of the beta-lactoglobulin gene may also be incorporated in constructs, as may genomic regions of the gene to be expressed. It is generally accepted in 15 the art that constructs lacking introns, for example, express poorly in comparison with those that contain such DNA sequences (see Brinster et al., Proc. Natl. Acad. Sci. USA 85: 836-840, 1988; Palmiter et al., Proc. Natl. Acad. Sci. USA 88: 478-482, 1991; Whitelaw et al., Transgenic Res. 1: 3-13, 1991; WO 89/01343; WO 91/02318). In this regard, it is generally preferred, where possible, to use genomic sequences containing all or some of the native 20 introns of a gene encoding the protein or polypeptide of interest. Within certain embodiments of the invention, 25 the further inclusion of at least some introns from the beta-lactoglobulin gene is preferred. One such region is a DNA segment which provides for intron splicing and RNA polyadenylation from the 3' non-coding region of the ovine beta-lactoglobulin gene. When substituted for the natural 30 3' non-coding sequences of a gene, this ovine beta-lactoglobulin segment can both enhance and stabilize expression levels of the protein or polypeptide of interest. Within other embodiments, the region surrounding the initiation ATG of one or more of the 35 fibrinogen sequences is replaced with corresponding sequences from a milk specific protein gene. Such replacement provides a putative tissue-specific initiation

environment to enhance expression. It is convenient to replace the entire fibrinogen chain pre-pro and 5' non-coding sequences with those of, for example, the BLG gene, although smaller regions may be replaced.

5 For expression of fibrinogen, DNA segments encoding each of the three component polypeptide chains of fibrinogen are operably linked to additional DNA segments required for their expression to produce expression units. Such additional segments include the above-mentioned milk
10 protein gene promoter, as well as sequences which provide for termination of transcription and polyadenylation of mRNA. The expression units will further include a DNA segment encoding a secretion signal operably linked to the segment encoding the fibrinogen polypeptide chain. The
15 secretion signal may be a native fibrinogen secretion signal or may be that of another protein, such as a milk protein. The term "secretion signal" is used herein to denote that portion of a protein that directs it through the secretory pathway of a cell to the outside. Secretion
20 signals are most commonly found at the amino-termini of proteins. See, for example, von Heinje, Nuc. Acids Res. 14: 4683-4690, 1986; and Meade et al., U.S. Patent No. 4,873,316, which are incorporated herein by reference.

Construction of expression units is conveniently
25 carried out by inserting a fibrinogen chain sequence into a plasmid or phage vector containing the additional DNA segments, although the expression unit may be constructed by essentially any sequence of ligations. It is particularly convenient to provide a vector containing a
30 DNA segment encoding a milk protein and to replace the coding sequence for the milk protein with that of a fibrinogen chain (including a secretion signal), thereby creating a gene fusion that includes the expression control sequences of the milk protein gene. In any event,
35 cloning of the expression units in plasmids or other vectors facilitates the amplification of the fibrinogen sequences. Amplification is conveniently carried out in

bacterial (e.g. *E. coli*) host cells, thus the vectors will typically include an origin of replication and a selectable marker functional in bacterial host cells.

In view of the size of the fibrinogen chain genes it is most practical to prepare three separate expression units, mix them, and introduce the mixture into the host. However, those skilled in the art will recognize that other protocols may be followed. For example, expression units for the three chains can be introduced individually into different embryos to be combined later by breeding. In a third approach, the three expression units can be linked in a single suitable vector, such as a yeast artificial chromosome or phage P1 clone. Coding sequences for two or three chains can be combined in polycistronic expression units (see, e.g., Levinson et al., U.S. Patent No. 4,713,339).

The expression unit(s) is(are) then introduced into fertilized eggs (including early-stage embryos) of the chosen host species. Introduction of heterologous DNA can be accomplished by one of several routes, including microinjection (e.g. U.S. Patent No. 4,873,191), retroviral infection (Jaenisch, Science 240: 1468-1474, 1988) or site-directed integration using embryonic stem (ES) cells (reviewed by Bradley et al., Bio/Technology 10: 534-539, 1992). The eggs are then implanted into the oviducts or uteri of pseudopregnant females and allowed to develop to term. Offspring carrying the introduced DNA in their germ line can pass the DNA on to their progeny in the normal, Mendelian fashion, allowing the development of transgenic herds. General procedures for producing transgenic animals are known in the art. See, for example, Hogan et al., Manipulating the Mouse Embryo: A Laboratory Manual, Cold Spring Harbor Laboratory, 1986; Simons et al., Bio/Technology 6: 179-183, 1988; Wall et al., Biol. Reprod. 32: 645-651, 1985; Buhler et al., Bio/Technology 8: 140-143, 1990; Ebert et al., Bio/Technology 9: 835-838, 1991; Krimpenfort et al.,

Bio/Technology 9: 844-847, 1991; Wall et al., J. Cell. Biochem. 49: 113-120, 1992; and WIPO publications WO 88/00239, WO 90/05188, WO 92/11757; and GB 87/00458, which are incorporated herein by reference. Techniques for 5 introducing foreign DNA sequences into mammals and their germ cells were originally developed in the mouse. See, e.g., Gordon et al., Proc. Natl. Acad. Sci. USA 77: 7380-7384, 1980; Gordon and Ruddle, Science 214: 1244-1246, 1981; Palmiter and Brinster, Cell 41: 343-345, 1985; 10 Brinster et al., Proc. Natl. Acad. Sci. USA 82: 4438-4442, 1985; and Hogan et al. (ibid.). These techniques were subsequently adapted for use with larger animals, including livestock species (see e.g., WIPO publications WO 88/00239, WO 90/05188, and WO 92/11757; and Simons et 15 al., Bio/Technology 6: 179-183, 1988). To summarize, in the most efficient route used to date in the generation of transgenic mice or livestock, several hundred linear molecules of the DNA of interest are injected into one of the pro-nuclei of a fertilized egg. Injection of DNA into 20 the cytoplasm of a zygote can also be employed.

It is preferred to obtain a balanced expression of each fibrinogen chain to allow for efficient formation of the mature protein. Ideally, the three expression units should be on the same DNA molecule for introduction 25 into eggs. This approach, however, may generate technical problems at, for example, the injection and manipulation stages. For example, the size of fibrinogen expression units may necessitate the use of yeast artificial chromosomes (YACs) or phage P1 to amplify and manipulate 30 the DNA prior to injection. If this approach is followed, segments of DNA to be injected, containing all three expression units, would be very large, thus requiring modification of the injection procedure using, for example, larger bore needles. In a more simple approach, 35 a mixture of each individual expression unit is used. It is preferred to combine equimolar amounts of the three expression units, although those skilled in the art will

recognize that this ratio may be varied to compensate for the characteristics of a given expression unit. Some expression, generally a reduced level, will be obtained when lesser molar amounts of one or two chains are used, 5 and expression efficiencies can generally be expected to decline in approximate proportion to the divergence from the preferred equimolar ratio. In any event, it is preferred to use a mixture having a ratio of $\text{A}\alpha:\text{B}\beta:\gamma$ expression units in the range of 0.5-1:0.5-1:0.5-1. When 10 the ratio is varied from equimolar, it is preferred to employ relatively more of the $\text{B}\beta$ expression unit. Alternatively, one or a mixture of two of the expression units is introduced into individual eggs. However, animals derived by this approach will express only one or 15 two fibrinogen chains. To generate an intact fibrinogen molecule by this approach requires a subsequent breeding program designed to combine all three expression units in individuals of a group of animals.

In general, female animals are superovulated by 20 treatment with follicle stimulating hormone, then mated. Fertilized eggs are collected, and the heterologous DNA is injected into the eggs using known methods. See, for example, U.S. Patent No. 4,873,191; Gordon et al., Proc. Natl. Acad. Sci. USA 77: 7380-7384, 1980; Gordon and 25 Ruddle, Science 214: 1244-1246, 1981; Palmiter and Brinster, Cell 41: 343-345, 1985; Brinster et al., Proc. Natl. Acad. Sci. USA 82: 4438-4442, 1985; Hogan et al., Manipulating the Mouse Embryo: A Laboratory Manual, Cold Spring Harbor Laboratory, 1986; Simons et al. 30 Bio/Technology 6: 179-183, 1988; Wall et al., Biol. Reprod. 32: 645-651, 1985; Buhler et al., Bio/Technology 8: 140-143, 1990; Ebert et al., Bio/Technology 9: 835-838, 1991; Krimpenfort et al., Bio/Technology 9: 844-847, 1991; Wall et al., J. Cell. Biochem. 49: 113-120, 1992; WIPO 35 publications WO 88/00239, WO 90/05118, and WO 92/11757; and GB 87/00458, which are incorporated herein by reference.

For injection into fertilized eggs, the expression units are removed from their respective vectors by digestion with appropriate restriction enzymes. For convenience, it is preferred to design the vectors so that

5 the expression units are removed by cleavage with enzymes that do not cut either within the expression units or elsewhere in the vectors. The expression units are recovered by conventional methods, such as electro-elution followed by phenol extraction and ethanol precipitation,

10 sucrose density gradient centrifugation, or combinations of these approaches.

DNA is injected into eggs essentially as described in Hogan et al., ibid. In a typical injection, eggs in a dish of an embryo culture medium are located

15 using a stereo zoom microscope (x50 or x63 magnification preferred). Suitable media include Hepes (N-2-hydroxyethylpiperazine-N'-2-ethanesulphonic acid) or bicarbonate buffered media such as M2 or M16 (available from Sigma Chemical Co., St. Louis, USA) or synthetic

20 oviduct medium (disclosed below). The eggs are secured and transferred to the center of a glass slide on an injection rig using, for example, a drummond pipette complete with capillary tube. Viewing at lower (e.g. x4) magnification is used at this stage. Using the holding

25 pipette of the injection rig, the eggs are positioned centrally on the slide. Individual eggs are sequentially secured to the holding pipette for injection. For each injection process, the holding pipette/egg is positioned in the center of the viewing field. The injection needle

30 is then positioned directly below the egg. Preferably using x40 Nomarski objectives, both manipulator heights are adjusted to focus both the egg and the needle. The pronuclei are located by rotating the egg and adjusting the holding pipette assembly as necessary. Once the

35 pronucleus has been located, the height of the manipulator is altered to focus the pronuclear membrane. The injection needle is positioned below the egg such that the

needle tip is in a position below the center of the pronucleus. The position of the needle is then altered using the injection manipulator assembly to bring the needle and the pronucleus into the same focal plane. The 5 needle is moved, via the joy stick on the injection manipulator assembly, to a position to the right of the egg. With a short, continuous jabbing movement, the pronuclear membrane is pierced to leave the needle tip inside the pronucleus. Pressure is applied to the 10 injection needle via the glass syringe until the pronucleus swells to approximately twice its volume. At this point, the needle is slowly removed. Reverting to lower (e.g. x4) magnification, the injected egg is moved to a different area of the slide, and the process is 15 repeated with another egg.

After the DNA is injected, the eggs may be cultured to allow the pronuclei to fuse, producing one-cell or later stage embryos. In general, the eggs are cultured at approximately the body temperature of the 20 species used in a buffered medium containing balanced salts and serum. Surviving embryos are then transferred to pseudopregnant recipient females, typically by inserting them into the oviduct or uterus, and allowed to develop to term. During embryogenesis, the injected DNA 25 integrates in a random fashion in the genomes of a small number of the developing embryos.

Potential transgenic offspring are screened via blood samples and/or tissue biopsies. DNA is prepared from these samples and examined for the presence of the 30 injected construct by techniques such as polymerase chain reaction (PCR; see Mullis, U.S. Patent No. 4,683,202) and Southern blotting (Southern, J. Mol. Biol. 98:503, 1975; Maniatis et al., Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, 1982). Founder transgenic 35 animals, or G0s, may be wholly transgenic, having transgenes in all of their cells, or mosaic, having transgenes in only a subset of cells (see, for example,

Wilkie et al., Develop. Biol. 118: 9-18, 1986). In the latter case, groups of germ cells may be wholly or partially transgenic. In the latter case, the number of transgenic progeny from a founder animal will be less than 5 the expected 50% predicted from Mendelian principles. Founder G0 animals are grown to sexual maturity and mated to obtain offspring, or G1s. The G1s are also examined for the presence of the transgene to demonstrate transmission from founder G0 animals. In the case of male 10 G0s, these may be mated with several non-transgenic females to generate many offspring. This increases the chances of observing transgene transmission. Female G0 founders may be mated naturally, artificially inseminated or superovulated to obtain many eggs which are transferred 15 to surrogate mothers. The latter course gives the best chance of observing transmission in animals having a limited number of young. The above-described breeding procedures are used to obtain animals that can pass the DNA on to subsequent generations of offspring in the 20 normal, Mendelian fashion, allowing the development of, for example, colonies (mice), flocks (sheep), or herds (pigs, goats and cattle) of transgenic animals.

The milk from lactating G0 and G1 females is examined for the expression of the heterologous protein 25 using immunological techniques such as ELISA (see Harlow and Lane, Antibodies, A Laboratory Manual, Cold Spring Harbor Laboratory, 1988) and Western blotting (Towbin et al., Proc. Natl. Acad. Sci. USA 76: 4350-4354, 1979). For a variety of reasons known in the art, expression levels 30 of the heterologous protein will be expected to differ between individuals.

A satisfactory family of animals should satisfy three criteria: they should be derived from the same founder G0 animal; they should exhibit stable transmission 35 of the transgene; and they should exhibit stable expression levels from generation to generation and from lactation to lactation of individual animals. These

principles have been demonstrated and discussed (Carver et al., Bio/Technology 11: 1263-1270, 1993). Animals from such a suitable family are referred to as a "line." Initially, male animals, G0 or G1, are used to derive a 5 flock or herd of producer animals by natural or artificial insemination. In this way, many female animals containing the same transgene integration event can be quickly generated from which a supply of milk can be obtained.

The fibrinogen is recovered from milk using 10 standard practices such as skimming, precipitation, filtration and protein chromatography techniques.

Fibrinogen produced according to the present invention is useful within human and veterinary medicine, such as in the formulation of surgical adhesives. 15 Adhesives of this type are known in the art. See, for example, U.S. Patents No. 4,377,572; 4,442,655; 4,462,567; and 4,627,879, which are incorporated herein by reference. In general, fibrinogen and factor XIII are combined to form a first component that is mixed just prior to use 20 with a second component containing thrombin. The thrombin converts the fibrinogen to fibrin, causing the mixture to gel, and activates the factor XIII. The activated factor XIII cross links the fibrin to strengthen and stabilize the adhesive matrix. Such adhesives typically contain 25 from about 30 mg/ml to about 100 mg/ml fibrinogen and from about 50 μ g/ml to about 500 μ g/ml factor XIII. They may also contain additional ingredients, such as aprotinin, albumin, fibronectin, bulking agents, and solubilizers. Methods for producing factor XIII are known in the art. 30 See, for example, U.S. Patent No. 5,204,447. The fibrinogen is also useful for coating surfaces of polymeric articles, e.g. synthetic vascular grafts, as disclosed in U.S. Patent No. 5,272,074 (incorporated herein by reference). 35 The invention is further illustrated by the following non-limiting examples.

ExamplesExample I

The multiple cloning site of the vector pUC18
5 (Yanisch-Perron et al., Gene 33:103-119, 1985) was removed
and replaced with a synthetic double stranded
oligonucleotide (the strands of which are shown in SEQ ID
NO: 8 and SEQ ID NO: 27) containing the restriction sites
Pvu I/Mlu I/Eco RV/Xba I/Pvu I/Mlu I, and flanked by 5'
10 overhangs compatible with the restriction sites Eco RI and
Hind III. pUC18 was cleaved with both Eco RI and Hind
III, the 5' terminal phosphate groups were removed with
calf intestinal phosphatase, and the oligonucleotide was
ligated into the vector backbone. The DNA sequence across
15 the junction was confirmed by sequencing, and the new
plasmid was called pUCPM.

The β -lactoglobulin (BLG) gene sequences from
pSS1tgXS (disclosed in WIPO publication WO 88/00239) were
excised as a Sal I-Xba I fragment and recloned into the
20 vector pUCPM that had been cut with Sal I and Xba I to
construct vector pUCXS. pUCXS is thus a pUC18 derivative
containing the entire BLG gene from the Sal I site to the
Xba I site of phage SS1 (Ali and Clark, J. Mol. Biol. 199:
415-426, 1988).

25 The plasmid pSS1tgSE (disclosed in WIPO
publication WO 88/00239) contains a 1290 bp BLG fragment
flanked by Sph I and EcoR I restriction sites, a region
spanning a unique Not I site and a single Pvu II site
which lies in the 5' untranslated leader of the BLG mRNA.
30 Into this Pvu II site was ligated a double stranded, 8 bp
DNA linker (5'-GGATATCC-3') encoding the recognition site
for the enzyme Eco RV. This plasmid was called
pSS1tgSE/RV. DNA sequences bounded by Sph I and Not I
restriction sites in pSS1tgSE/RV were excised by enzymatic
35 digestion and used to replace the equivalent fragment in
pUCXS. The resulting plasmid was called pUCXSRV. The
sequence of the BLG insert in pUCXSRV is shown in SEQ ID

NO: 7, with the unique Eco RV site at nucleotide 4245 in the 5' untranslated leader region of the BLG gene. This site allows insertion of any additional DNA sequences under the control of the BLG promoter 3' to the 5 transcription initiation site.

Using the primers BLGAMP3 (5'-TGG ATC CCC TGC CGG TGC CTC TGG-3'; SEQ ID NO: 9) and BLGAMP4 (5'-AAC GCG TCA TCC TCT GTG AGC CAG-3'; SEQ ID NO: 10) a PCR fragment of approximately 650 bp was produced from sequences 10 immediately 3' to the stop codon of the BLG gene in pUCXSRV. The PCR fragment was engineered to have a BamH I site at its 5' end and an Mlu I site at its 3' end and was cloned as such into BamH I and Mlu I cut pGEM7zf(+) (Promega) to give pDAM200(+).

15 pUCXSRV was digested with Kpn I, and the largest, vector containing band was gel purified. This band contained the entire pUC plasmid sequences and some 3' non-coding sequences from the BLG gene. Into this backbone was ligated the small Kpn I fragment from 20 pDAM200(+) which, in the correct orientation, effectively engineered a BamH I site at the extreme 5' end of the 2.6 Kbp of the BLG 3' flanking region. This plasmid was called pBLAC200. A 2.6 Kbp Cla I-Xba I fragment from pBLAC200 was ligated into Cla I-Xba I cut pSP72 vector 25 (Promega), thus placing an EcoR V site immediately upstream of the BLG sequences. This plasmid was called pBLAC210.

30 The 2.6 Kbp Eco RV-Xba I fragment from pBLAC210 was ligated into Eco RV-Xba I cut pUCXSRV to form pMAD6. This, in effect, excised all coding and intron sequences from pUCXSRV, forming a BLG minigene consisting of 4.3 Kbp of 5' promoter and 2.6 Kbp of 3' downstream sequences flanking a unique EcoR V site. An oligonucleotide linker (ZC6839: ACTACGTAGT; SEQ ID NO: 11) was inserted into the 35 Eco RV site of pMAD6. This modification destroyed the Eco RV site and created a Sna BI site to be used for cloning purposes. The vector was designated pMAD6-Sna. Messenger

RNA initiates upstream of the Sna BI site and terminates downstream of the Sna BI site. The precursor transcript will encode a single BLG-derived intron, intron 6, which is entirely within the 3' untranslated region of the gene.

5

Example II

Clones encoding the individual fibrinogen chains were obtained from the laboratory of Dr. Earl W. Davie, 10 University of Washington, Seattle. A genomic fibrinogen $\text{A}\alpha$ -chain clone (Chung et al., 1990, *ibid.*) was obtained from the plasmid BS4. This plasmid contains the $\text{A}\alpha$ clone inserted into the Sal I and Bam HI sites of the vector pUC18, but lacks the coding sequence for the first four 15 amino acids of the $\text{A}\alpha$ chain. A genomic $\text{B}\beta$ -chain DNA (Chung et al., *ibid.*) was isolated from a lambda Charon 4A phage clone (designated $\beta\lambda 4$) as two EcoRI fragments of ca. 5.6 Kbp each. The two fragments were cloned separately into pUC19 that had been digested with Eco RI and treated with 20 calf intestinal phosphatase. The resulting clones were screened by digestion with the restriction enzyme *Pvu* II to distinguish plasmids with the 5' and 3' $\text{B}\beta$ inserts (designated Beta5'RI/puc and Beta3'RI/puc, respectively). Genomic γ -chain clones were isolated as described by Rixon 25 et al. (*Biochemistry* 24: 2077-2086, 1985). Clone $\text{p}\gamma 12\text{A}9$ comprises 5' non-coding sequences and approximately 4535 bp of γ -chain coding sequence. Clone $\text{p}\gamma 12\text{F}3$ comprises the remaining coding sequence and 3' non-coding nucleotides. Both are pBR322-based plasmids with the fibrinogen 30 sequences inserted at the EcoRI site. These plasmids were used as templates for the respective PCR reactions.

The fibrinogen chain coding sequences were tailored for insertion into expression vectors using the polymerase chain reaction (PCR) as generally described by 35 Mullis (U.S. Patent No. 4,683,202). This procedure removed native 5' and 3' untranslated sequences, added a 9 base sequence (CCT GCA GCC) upstream of the first ATG of

each coding sequence, supplied the first four codons for the $\text{A}\alpha$ -chain sequence, removed an internal Mlu I site in the $\text{A}\alpha$ sequence and added restriction sites to facilitate subsequent cloning steps.

5 Referring to Figure 1, the 5' end of the $\text{A}\alpha$ coding sequence was tailored in a PCR reaction containing 20 pmole for each of primers ZC6632 (SEQ ID NO: 12) and ZC6627 (SEQ ID NO: 13), approximately 10 ng of plasmid BS4 template DNA, 10 μl of a mix containing 2.5 mM each dNTP, 10 7.5 μl 10x *Pyrococcus furiosus* (Pfu) DNA polymerase buffer #1 (200 mM Tris-HCl, pH 8.2, 100 mM KCl, 60 mM $(\text{NH}_4)_2\text{SO}_4$, 20 mM MgCl_2 , 1% Triton X-100, 100 $\mu\text{g}/\text{ml}$ nuclease free bovine serum albumin) (Stratagene, La Jolla, CA), and water to 75 μl . The mixture was heated to 94°C in a DNA thermal 15 cycler (Perkin-Elmer Corp., Norwalk, CT). To the heated mixture was added 25 μl of a mixture containing 2.5 μl 10x Pfu buffer #1, 22 μl H_2O and 1 μl 2.5 units/ μl Pfu DNA polymerase (Stratagene). The reactions were run in a DNA thermal cycler (Perkin-Elmer) for five cycles of 94°, 45 20 seconds; 40°, 90 seconds; 72°, 120 seconds; 20 cycles of 94°, 45 seconds; 45°, 90 seconds; 72°, 120 seconds; then incubated at 72° for 7 minutes. The 5' PCR-generated fragment was digested with Bam HI and Hind III , and the Bam HI-Hind III fragment was then ligated to an internal 25 2.91 Kbp Hind III-Xba I fragment and Bam HI , Xba I -digested pUC18. PCR-generated exon sequences were sequenced.

Referring again to Figure 1, the 3' end of the $\text{A}\alpha$ coding sequence was tailored in a series of steps in 30 which the Mlu I site 563 bases upstream from the stop codon of the $\text{A}\alpha$ sequence was mutated using an overlap extension PCR reaction (Ho et al., *Gene* 77: 51-59, 1989). In the first reaction 40 pmole of each of primers ZC6521 (SEQ ID NO: 14) and ZC6520 (SEQ ID NO: 15) were combined 35 with approximately 10 ng of plasmid BS4 template DNA in a reaction mixture as described above. The reaction was run for 5 cycles of 94°, 45 seconds; 40°, 60 seconds; 72°, 120

seconds; 15 cycles of 94°, 45 seconds; 45°, 60 seconds; 72°, 120 seconds; then incubated at 72° for 7 minutes. A second reaction was carried out in the same manner using 40 pmole of each of primers ZC6519 (SEQ ID NO: 16) and 5 ZC6518 (SEQ ID NO: 17) and BS4 as template. The PCR-generated DNA fragments from the first and second reactions were isolated by gel electrophoresis and elution from the gel. Approximately 1/10 of each recovered reaction product was combined with 40 pmole of each of 10 primers ZC6521 (SEQ ID NO: 14) and ZC6518 (SEQ ID NO: 17) in a PCR reaction in which the complementary 3' ends of each fragment (containing the single base change) annealed and served as a primer for the 3' extension of the complementary strand. PCR was carried out using the same 15 reaction conditions as in the first and second 3' PCR steps. The reaction product was then digested with Xba I and Bam HI, and the Xba I-Bam HI fragment was cloned into Xba I, Bam HI-digested pUC18. PCR-generated exons were sequenced.

20 As shown in Figure 1, the 5' Bam HI-Xba I fragment (3.9 Kbp) and the 3' Xba I-Bam HI fragment (1.3 Kbp) were inserted into the Bam HI site of the vector Zem228. Zem228 is a pUC18 derivative comprising a Bam HI cloning site between a mouse MT-1 promoter and SV40 25 terminator, and a neomycin resistance marker flanked by SV40 promoter and terminator sequences. See European Patent Office Publication EP 319,944 and Fig. 2. The entire A α coding sequence was isolated from the Zem228 vector as an Sna BI fragment, which was inserted into the 30 Sna BI site of the plasmid pMAD6-Sna.

Referring to Fig. 3, the 5' end of the B β -chain was tailored by PCR using the oligonucleotides ZC6629 (SEQ ID NO: 18), ZC6630 (SEQ ID NO: 19) and ZC6625 (SEQ ID NO: 20). These primers were used in pairwise combinations 35 (ZC6629 + ZC6625 or ZC6630 + ZC6625) to generate B β coding sequences beginning at the first ATG codon (position 470 in SEQ ID NO: 3) (designated N1-Beta) or the third ATG

codon (position 512 in SEQ ID NO: 3) (designated N3-Beta). Approximately 5 ng of Beta5'RI/puc template DNA was combined with 20 pmole of each of the primers (N1-Beta:ZC6629, SEQ ID NO: 18 + ZC6625, SEQ ID NO: 20; or N3-Beta:ZC6630, SEQ ID NO: 19 + ZC6625, SEQ ID NO: 20) in a reaction mixture as described above. The mixtures were incubated for 5 cycles of 94°, 45 seconds; 40°, 120 seconds; (N1-Beta) or 90 seconds (N3-Beta); 72°, 120 seconds; 20 cycles of 94°, 45 seconds; 45°, 120 seconds; 10 (N1-Beta) or 90 seconds (N3-Beta); 72°, 120 seconds; then incubated at 72° for 7 minutes. The two reaction products N1, 555 bp or N3, 510 bp) were each digested with Eco RI and Bgl II, and the fragments were ligated to the internal Bgl II-Xba I fragment and Eco RI + Xba I-digested pUC19. 15 The 3' end of the B β sequence was tailored in a reaction mixture as described above using the oligonucleotide primers ZC6626 (SEQ ID NO: 21) and ZC6624 (SEQ ID NO: 22) and approximately 5 ng of Beta3'RI/puc template. The mixtures were incubated for 5 cycles of 94°, 45 seconds; 20 40°, 90 seconds; 72°, 120 seconds; 15 cycles of 94°, 45 seconds; 45°, 90 seconds; 72°, 120 seconds; then incubated at 72° for 7 minutes. A 990 bp Bgl II-Eco RI fragment was isolated. This 3' fragment was ligated to the adjacent coding fragment (340 bp, Sph I-Bgl II) and Sph I + Eco RI-digested pUC19. The 3' and 5' PCR-generated exons were 25 sequenced. A third intermediate vector was constructed by combining two internal fragments (4285 bp Xba I-Eco RI and 383 kb Eco RI-Sph I) in Xba I + Sph I-digested pUC19. The entire B β coding sequence (two forms) was then assembled 30 by ligating one of the 5' Eco RI-Xba I fragments, the internal Xba I-Sph I fragment, the 3' Sph I-Eco RI fragment and Eco RI-digested vector pUC19. The B β sequence was then isolated as a 7.6 Kbp Sna BI fragment and inserted into the Sna BI site of pMAD6-Sna. 35 Referring to Fig. 4, the 5' end of the gamma chain sequence was tailored by PCR using the oligonucleotide primers ZC6514 (SEQ ID NO: 23) and ZC6517

(SEQ ID NO: 24) and approximately 50 ng of p γ 12A9 as template. The PCR reaction was run as described above using 40 pM of each primer. The reaction was run for 5 cycles of 94°, 45 seconds; 40°, 60 seconds, 72°, 120 seconds, followed by 15 cycles of 94°, 45 seconds; 45°, 60 seconds; 72°, 120 seconds. The resulting 213 bp fragment was digested with Bam HI and Spe I, and the resulting restriction fragment was ligated with the adjacent downstream 4.4 kb Spe I-Eco RI fragment and Bam HI + Eco RI digested pUC19. The 3' end of the gamma chain sequence was tailored using oligonucleotide primers ZC6516 (SEQ ID NO: 25) and ZC6515 (SEQ ID NO: 26) using 40 pM of each primer, approximately 50 ng of p γ 12F3 template and the same thermal cycling schedule as used for the 5' fragment.

15 The resulting 500 bp fragment was digested with Spe I and Bam HI, and the resulting restriction fragment was ligated with the upstream 2.77 kb Eco RI-Spe I fragment and Eco RI + Bam HI-digested pUC19. All PCR-generated exons were sequenced. The entire γ' -chain coding sequence was then

20 assembled by ligating a 4.5 Kbp Bam HI-Eco RI 5' fragment, a 1.1 Kbp Eco RI-Pst I internal fragment and a 2.14 Kbp Pst I-Xba I 3' fragment in Bam HI + Xba I-digested Zem219b. Zem219b is a pUC18-derived vector containing a mouse metallothionein promoter and a DHFR selectable

25 marker operably linked to an SV40 promoter (Fig. 5). Plasmid Zem219b has been deposited with American Type Culture Collection as an *E. coli* XL1-blue transformant under Accession No. 68979. The entire γ' -chain coding sequence was then isolated as a 7.8 Kbp Sna B1 fragment

30 and inserted into the Sna B1 site of pMAD6-Sna.

Example III

Mice for initial breeding stocks (C57BL6J, CBACA) were obtained from Harlan Olac Ltd. (Bicester, UK).
35 These were mated in pairs to produce F1 hybrid cross (B6CBAF1) for recipient female, superovulated females, stud males and vasectomized males. All animals were kept

on a 14 hour light/10 hour dark cycle and fed water and food (Special Diet Services RM3, Edinburgh, Scotland) ad libitum.

Transgenic mice were generated essentially as 5 described in Hogan et al., Manipulating the Mouse Embryo: A Laboratory Manual, Cold Spring Harbor Laboratory, 1986, which is incorporated herein by reference in its entirety. Female B6CBAF1 animals were superovulated at 4-5 weeks of age by an i.p. injection of pregnant mares' serum 10 gonadotrophin (FOLLIGON, Vet-Drug, Falkirk, Scotland) (5 iu) followed by an i.p. injection of human chorionic gonadotrophin (CHORULON, Vet-Drug, Falkirk, Scotland) (5 iu) 45 hours later. They were then mated with a stud male overnight. Such females were next examined for copulation 15 plugs. Those that had mated were sacrificed, and their eggs were collected for microinjection.

DNA was injected into the fertilized eggs as described in Hogan et al. (ibid.) Briefly, each of the vectors containing the $A\alpha$, $B\beta$ and γ expression units was 20 digested with Mlu I, and the expression units were isolated by sucrose gradient centrifugation. All chemicals used were reagent grade (Sigma Chemical Co., St. Louis, MO, U.S.A.), and all solutions were sterile and nuclease-free. Solutions of 20% and 40% sucrose in 1 M 25 NaCl, 20 mM Tris pH 8.0, 5 mM EDTA were prepared using UHP water and filter sterilized. A 30% sucrose solution was prepared by mixing equal volumes of the 20% and 40% solutions. A gradient was prepared by layering 0.5 ml steps of the 40%, 30% and 20% sucrose solutions into a 2 30 ml polyallomer tube and allowed to stand for one hour. 100 μ l of DNA solution (max. 8 μ g DNA) was loaded onto the top of the gradient, and the gradient was centrifuged for 17-20 hours at 26,000 rpm, 15°C in a Beckman TL100 ultracentrifuge using a TLS-55 rotor (Beckman Instruments, 35 Fullerton, CA, USA). Gradients were fractionated by puncturing the tube bottom with a 20 ga. needle and collecting drops in a 96 well microtiter plate. 3 μ l

aliquots were analyzed on a 1% agarose mini-gel. Fractions containing the desired DNA fragment were pooled and ethanol precipitated overnight at -20°C in 0.3M sodium acetate. DNA pellets were resuspended in 50-100 μ l UHP 5 water and quantitated by fluorimetry. The expression units were diluted in Dulbecco's phosphate buffered saline without calcium and magnesium (containing, per liter, 0.2 g KCl, 0.2 g KH_2PO_4 , 8.0 g NaCl, 1.15 g Na_2HPO_4), mixed (using either the N1-Beta or N3-Beta expression unit) in a 10 1:1:1 molar ratio, concentration adjusted to about 6 μ g/ml, and injected into the eggs (~2 μ l total DNA 15 solution per egg).

Recipient females of 6-8 weeks of age are prepared by mating B6CBAF1 females in natural estrus with 15 vasectomized males. Females possessing copulation plugs are then kept for transfer of microinjected eggs.

Following birth of potential transgenic animals, tail biopsies are taken, under anesthesia, at four weeks of age. Tissue samples are placed in 2 ml of tail buffer 20 (0.3 M Na acetate, 50 mM HCl, 1.5 mM MgCl_2 , 10 mM Tris-HCl, pH 8.5, 0.5% NP40, 0.5% Tween 20) containing 200 μ g/ml proteinase K (Boehringer Mannheim, Mannheim, Germany) and vortexed. The samples are shaken (250 rpm) at 55°-60° for 3 hours to overnight. DNA prepared from 25 biopsy samples is examined for the presence of the injected constructs by PCR and Southern blotting. The digested tissue is vigorously vortexed, and 5 μ l aliquots are placed in 0.5 ml microcentrifuge tubes. Positive and negative tail samples are included as controls. Forty μ l 30 of silicone oil (BDH, Poole, UK) is added to each tube, and the tubes are briefly centrifuged. The tubes are incubated in the heating block of a thermal cycler (e.g. Omni-gene, Hybaid, Teddington, UK) to 95°C for 10 minutes. Following this, each tube has a 45 μ l aliquot of PCR mix 35 added such that the final composition of each reaction mix is: 50 mM KCl; 2 mM MgCl_2 ; 10 mM Tris-HCl (pH 8.3); 0.01% gelatin; 0.1% NP40, 10% DMSO; 500 nM each primer, 200 μ M

dNTPs; 0.02 U/ μ l Taq polymerase (Boehringer Mannheim, Mannheim, Germany). The tubes are then cycled through 30 repeated temperature changes as required by the particular primers used. The primers may be varied but in all cases 5 must target the BLG promoter region. This is specific for the injected DNA fragments because the mouse does not have a BLG gene. Twelve μ l of 5x loading buffer containing Orange G marker dye (0.25% Orange G [Sigma] 15% Ficoll type 400 [Pharmacia Biosystems Ltd., Milton Keynes, UK]) 10 is then added to each tube, and the reaction mixtures are electrophoresed on a 1.6% agarose gel containing ethidium bromide (Sigma) until the marker dye has migrated 2/3 of the length of the gel. The gel is visualized with a UV light source emitting a wavelength of 254 nm. Transgenic 15 mice having one or more of the injected DNA fragments are identified by this approach.

Positive tail samples are processed to obtain pure DNA. The DNA samples are screened by Southern blotting using a BLG promoter probe (nucleotides 2523-4253 20 of SEQ ID NO: 7). Specific cleavages with appropriate restriction enzymes (e.g. Eco RI) allow the distinction of the three constructs containing the α , β and γ sequences.

Southern blot analysis of transgenic mice prepared essentially as described above demonstrated that 25 more than 50% of progeny contained all three fibrinogen sequences. Examination of milk from positive animals by reducing SDS polyacrylamide gel electrophoresis demonstrated the presence of all three protein chains at concentrations up to 1 mg/ml. The amount of fully 30 assembled fibrinogen was related to the ratios of individual subunits present in the milk. No apparent phenotype was associated with high concentrations of human fibrinogen in mouse milk.

35 Example IV

Donor ewes are treated with an intravaginal progesterone-impregnated sponge (CHRONOGEST Goat Sponge,

Intervet, Cambridge, UK) on day 0. Sponges are left in situ for ten or twelve days.

Superovulation is induced by treatment of donor ewes with a total of one unit of ovine follicle stimulating hormone (OFSH) (OVAGEN, Horizon Animal Reproduction Technology Pty. Ltd., New Zealand) administered in eight intramuscular injections of 0.125 units per injection starting at 5:00 pm on day -4 and ending at 8:00 am on day 0. Donors are injected 10 intramuscularly with 0.5 ml of a luteolytic agent (ESTRUMATE, Vet-Drug) on day -4 to cause regression of the corpus luteum, to allow return to estrus and ovulation. To synchronize ovulation, the donor animals are injected intramuscularly with 2 ml of a synthetic releasing hormone 15 analog (RECEPTAL, Vet-Drug) at 5:00 pm on day 0.

Donors are starved of food and water for at least 12 hours before artificial insemination (A.I.). The animals are artificially inseminated by intrauterine laparoscopy under sedation and local anesthesia on day 1. 20 Either xylazine (ROMPUN, Vet-Drug) at a dose rate of 0.05-0.1 ml per 10 kg bodyweight or ACP injection 10 mg/ml (Vet-Drug) at a dose rate of 0.1 ml per 10 kg bodyweight is injected intramuscularly approximately fifteen minutes before A.I. to provide sedation. A.I. is carried out 25 using freshly collected semen from a Poll Dorset ram. Semen is diluted with equal parts of filtered phosphate buffered saline, and 0.2 ml of the diluted semen is injected per uterine horn. Immediately pre- or post-A.I., donors are given an intramuscular injection of AMOXYPEN 30 (Vet-Drug).

Fertilized eggs are recovered on day 2 following starvation of donors of food and water from 5:00 pm on day 1. Recovery is carried out under general anesthesia induced by an intravenous injection of 5% thiopentone 35 sodium (INTRAVAL SODIUM, Vet-Drug) at a dose rate of 3 ml per 10 kg bodyweight. Anesthesia is maintained by inhalation of 1-2% Halothane/O₂/N₂O after intubation. To

recover the fertilized eggs, a laparotomy incision is made, and the uterus is exteriorized. The eggs are recovered by retrograde flushing of the oviducts with Ovum Culture Medium (Advanced Protein Products, Brierly Hill, 5 West Midlands, UK) supplemented with bovine serum albumin of New Zealand origin. After flushing, the uterus is returned to the abdomen, and the incision is closed. Donors are allowed to recover post-operatively or are euthanized. Donors that are allowed to recover are given 10 an intramuscular injection of Amoxypen L.A. at the manufacturer's recommended dose rate immediately pre- or post-operatively.

Plasmids containing the three fibrinogen chain expression units are digested with Mlu I, and the 15 expression unit fragments are recovered and purified on sucrose density gradients. The fragment concentrations are determined by fluorimetry and diluted in Dulbecco's phosphate buffered saline without calcium and magnesium as described above. The concentration is adjusted to 6 μ g/ml 20 and approximately 2 pl of the mixture is microinjected into one pronucleus of each fertilized eggs with visible pronuclei.

All fertilized eggs surviving pronuclear microinjection are cultured in vitro at 38.5°C in an 25 atmosphere of 5% CO₂:5% O₂:90% N₂ and about -100% humidity in a bicarbonate buffered synthetic oviduct medium (see Table) supplemented with 20% v/v vasectomized ram serum. The serum may be heat inactivated at 56°C for 30 minutes and stored frozen at -20°C prior to use. The fertilized 30 eggs are cultured for a suitable period of time to allow early embryo mortality (caused by the manipulation techniques) to occur. These dead or arrested embryos are discarded. Embryos having developed to 5 or 6 cell divisions are transferred to synchronized recipient ewes.

Table
Synthetic Oviduct Medium

5	<u>Stock A (Lasts 3 Months)</u>	
	NaCl	6.29 g
	KCl	0.534 g
	KH ₂ PO ₄	0.162 g
10	MgSO ₄ .7H ₂ O	0.182 g
	Penicillin	0.06 g
	Sodium Lactate 60% syrup	0.6 mls
	Super H ₂ O	99.4 mls
15	<u>Stock B (Lasts 2 weeks)</u>	
	NaHCO ₃	0.21 g
	Phenol red	0.001 g
	Super H ₂ O	10 mls
20	<u>Stock C (Lasts 2 weeks)</u>	
	Sodium Pyruvate	0.051 g
	Super H ₂ O	10 mls
25	<u>Stock D (Lasts 3 months)</u>	
	CaCl ₂ .2H ₂ O	0.262 g
	Super H ₂ O	10 mls
30	<u>Stock E (Lasts 3 months)</u>	
	Hepes	0.651 g
	Phenol red	0.001 g
	Super H ₂ O	10 mls
35	<u>To make up 10mls of Bicarbonate Buffered Medium</u>	
	STOCK A	1 ml
	STOCK B	1 ml
	STOCK C	0.07 ml
	STOCK D	0.1 ml
	Super H ₂ O	7.83 ml
40	Osmolarity should be 265-285 mOsm. Add 2.5 ml of heat inactivated sheep serum and filter sterilize.	
45	<u>To make up 10 mls of HEPES Buffered Medium</u>	
	STOCK A	1 ml
	STOCK B	0.2 ml
	STOCK C	0.07 ml
	STOCK D	0.1 ml
	STOCK E	0.8 ml
50	Super H ₂ O	7.83 ml

Table, cont.

5 Osmolarity should be 265-285 mOsm.
Add 2.5 ml of heat inactivated sheep serum
and filter sterilize.

Recipient ewes are treated with an intravaginal progesterone-impregnated sponge (Chronogest Ewe Sponge or Chronogest Ewe-Lamb Sponge, Intervet) left in situ for 10 or 12 days. The ewes are injected intramuscularly with 1.5 ml (300 iu) of a follicle stimulating hormone substitute (P.M.S.G., Intervet) and with 0.5 ml of a luteolytic agent (Estrumate, Coopers Pitman-Moore) at sponge removal on day -1. The ewes are tested for estrus with a vasectomized ram between 8:00 am and 5:00 pm on days 0 and 1.

Embryos surviving in vitro culture are returned to recipients (starved from 5:00 pm on day 5 or 6) on day 20 6 or 7. Embryo transfer is carried out under general anesthesia as described above. The uterus is exteriorized via a laparotomy incision with or without laparoscopy. Embryos are returned to one or both uterine horns only in ewes with at least one suitable corpora lutea. After 25 replacement of the uterus, the abdomen is closed, and the recipients are allowed to recover. The animals are given an intramuscular injection of Amoxypen L.A. at the manufacturer's recommended dose rate immediately pre- or post-operatively.

30 Lambs are identified by ear tags and left with their dams for rearing. Ewes and lambs are either housed and fed complete diet concentrates and other supplements and or ad lib. hay, or are let out to grass.

Within the first week of life (or as soon thereafter as possible without prejudicing health), each lamb is tested for the presence of the heterologous DNA by two sampling procedures. A 10 ml blood sample is taken from the jugular vein into an EDTA vacutainer. If fit enough, the lambs also have a second 10 ml blood sample

taken within one week of the first. Tissue samples are taken by tail biopsy as soon as possible after the tail has become desensitized after the application of a rubber elastrator ring to its proximal third (usually within 200 5 minutes after "tailing"). The tissue is placed immediately in a solution of tail buffer. Tail samples are kept at room temperature and analyzed on the day of collection. All lambs are given an intramuscular injection of Amoxypen L.A. at the manufacturer's 10 recommended dose rate immediately post-biopsy, and the cut end of the tail is sprayed with an antibiotic spray.

DNA is extracted from sheep blood by first separating white blood cells. A 10 ml sample of blood is diluted in 20 ml of Hank's buffered saline (HBS; obtained 15 from Sigma Chemical Co.). Ten ml of the diluted blood is layered over 5 ml of Histopaque (Sigma) in each of two 15 ml screw-capped tubes. The tubes are centrifuged at 3000 rpm (2000 x g max.), low brake for 15 minutes at room temperature. White cell interfaces are removed to a clean 20 15 ml tube and diluted to 15 ml in HBS. The diluted cells are spun at 3000 rpm for 10 minutes at room temperature, and the cell pellet is recovered and resuspended in 2-5 ml of tail buffer.

To extract DNA from the white cells, 10% SDS is 25 added to the resuspended cells to a final concentration of 1%, and the tube is inverted to mix the solution. One mg of fresh proteinase K solution is added, and the mixture is incubated overnight at 45°C. DNA is extracted using an equal volume of phenol/chloroform (x3) and 30 chloroform/isoamyl alcohol (x1). The DNA is then precipitated by adding 0.1 volume of 3 M NaOAc and 2 volumes of ethanol, and the tube is inverted to mix. The precipitated DNA is spooled out using a clean glass rod with a sealed end. The spool is washed in 70% ethanol, 35 and the DNA is allowed to partially dry, then is redissolved in TE (10 mM Tris-HCl, 1 mM EDTA, pH 7.4).

DNA samples from blood and tail are analyzed by Southern blotting using probes for the BLG promoter region and the fibrinogen chain coding regions.

From the foregoing, it will be appreciated that, 5 although specific embodiments of the invention have been described herein for purposes of illustration, various modifications may be made without deviating from the spirit and scope of the invention. Accordingly, the invention is not limited except as by the appended claims.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: ZymoGenetics, Inc.
1201 Eastlake Avenue East
Seattle, Washington 98102
United States of America

Pharmaceutical Proteins Ltd.
Roslin
Edinburgh
Midlothian, Scotland EH25 9PP

(ii) TITLE OF INVENTION: Production of Fibrinogen in Transgenic
Animals

(iii) NUMBER OF SEQUENCES: 27

(iv) CORRESPONDENCE ADDRESS:

(A) ADDRESSEE: ZymoGenetics, Inc.
(B) STREET: 1201 Eastlake Avenue East
(C) CITY: Seattle
(D) STATE: WA
(E) COUNTRY: USA
(F) ZIP: 98102

(v) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA:

(A) APPLICATION NUMBER:
(B) FILING DATE:
(C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:

(A) NAME: Parker, Gary E
(B) REGISTRATION NUMBER: 31-648
(C) REFERENCE/DOCKET NUMBER: 93-15PC

(ix) TELECOMMUNICATION INFORMATION:

(A) TELEPHONE: 206-442-6673
(B) TELEFAX: 206-442-6678

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 5943 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(vii) IMMEDIATE SOURCE:

- (B) CLONE: Human Fibrinogen A-alpha chain

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: join(31..84, 1154..1279, 1739..1922, 3055..3200, 3786..5210)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

GTCTAGGAGC CAGCCCCACC CTTAGAAAAG ATG TTT TCC ATG AGG ATC GTC TGC	54
Met Phe Ser Met Arg Ile Val Cys	
1 5	
CTA GTT CTA AGT GTG GTG GGC ACA GCA TGG GTATGGCCCT TTTCA	104
Leu Val Leu Ser Val Val Gly Thr Ala Trp	
10 15	
TCTTCTTGCT TTCTCTCTGG TGTTTATTCC ACAAAAGAGCC TGGAGGTCAG AGTCTACCTG	164
CTCTATGTCC TGACACACTC TTAGCTTTAT GACCCCAGGC CTGGGAGGAA ATTTCTGGG	224
TGGGCTTGAC ACCTCAAGAA TACAGGGTAA TATGACACCA AGAGGAAGAT CTTAGATGGA	284
TGAGAGTGTAA CAACTACAAG GGAAACTTTA GCATCTGTCA TTCAGTCTTA CCACATTTG	344
TTTTGTTTG TTTTAAAAAG GGCAAGAATT ATTTGCCATC CTTGTACCTA TAAAGCCTTG	404
GTGCATTATA ATGCTAGTTA ATGGAATAAA ACATTTATG GTAAGATTTG TTTCTTTAG	464
TTATTAATTT CTTGCTACTT GTCCATAATA AGCAGAACTT TTAGTGTAG TACAGTTTG	524
CTGAAAGGTT ATTGTTGTGT TTGTCAAGAC AGAAGAAAAA GCAAACGAAT TATCTTTGGA	584
AATATCTTG CAGTATCAGA AGAGATTAGT TAGTAAGGCA ATACGCTTTT CCGCAGTAAT	644

GGTATTCTT TAAATTATGA ATCCATCTCT AAAGGTTACA TAGAAACTTG AAGGAGAGAG	704	
GAACATTCAG TTAAGATAGT CTAGGTTTT CTACTGAAGC AGCAATTACA GGAGAAAGAG	764	
CTCTACAGTA GTTTCACT TTCTGTCTGC AGTCATTAGT AAAATGAAA AGTAAAATT	824	
TAAC TGATT TATAGATTCA AATAATTTC CTTTAGGAT GGATTCTTA AACTCCTAA	884	
TATTTATCAA ATGCTTATT AAGTGTACA CACAGTTAAG AAATTGTAC ACCTTGTCTC	944	
CTTTAATTCT CATAACAAC CCATAAAATG GGTCTAGGA TTTCCATTG AAGATAAGAA	1004	
ACCTGAAGCT TGCGGAAGCC CTGTGTCTGC TCTCCTTAAT CTCTGTGAGA GTGCCATCTC	1064	
TTCTGGGGA CTTGTAGGCA TGCCACTGTC TCCTCTCTG GCTAACATTG CTGTTGCTCT	1124	
CTTTTGTA TGTGAATGAA TCTTTAAAG ACT GCA GAT AGT GGT GAA GGT GAC	1177	
Thr Ala Asp Ser Gly Glu Gly Asp		
20	25	
TTT CTA GCT GAA GGA GGA GGC GTG CGT GGC CCA AGG GTT GTG GAA AGA	1225	
Phe Leu Ala Glu Gly Gly Val Arg Gly Pro Arg Val Val Glu Arg		
30	35	40
CAT CAA TCT GCC TGC AAA GAT TCA GAC TGG CCC TTC TGC TCT GAT GAA	1273	
His Gln Ser Ala Cys Lys Asp Ser Asp Trp Pro Phe Cys Ser Asp Glu		
45	50	55
GAC TGG GTAAGCAGTC AGCGGGGGAA GCAGGGAGATT CCTTCCCTCT GATGCTAGAG	1329	
Asp Trp		
60		
GGGCTCACAG GCTGACCTGA TTGGTCCCAG AAACCTTTT AAATAGAAAA TAATTGAATA	1389	
GTTACCTACA TAGCAAATAA AGAAAAGGAA CCTACTCCCA AGAGCACTGT TTATTTACCT	1449	
CCCCAACTCT GGATCATTAG TGGGTGAACA GACAGGATT CAGTTGCATG CTCAGGCAAA	1509	
ACCAGGCTCC TGAGTATTGT GGCCTCAATT TCCTGGCACC TATTTATGGC TAAGTGGACC	1569	
CTCATTCCAG AGTTTCTCTG CGACCTCTAA CTAGTCCCTCT TACCTACTTT TAAGCCAAC	1629	
TATCTGGAAG AGAAAGGGTA GGAAGAAATG GGGGCTGCAT GGAAACATGC AAAATTATTC	1689	
TGAATCTGAG AGATAGATCC TTACTGTAAT TTCTCCCTT CACTTCAAG AAC TAC	1744	
Asn Tyr		

AAA TGC CCT TCT GGC TGC AGG ATG AAA GGG TTG ATT GAT GAA GTC AAT Lys Cys Pro Ser Gly Cys Arg Met Lys Gly Leu Ile Asp Glu Val Asn 65 70 75	1792
CAA GAT TTT ACA AAC AGA ATA AAT AAG CTC AAA AAT TCA CTA TTT GAA Gln Asp Phe Thr Asn Arg Ile Asn Lys Leu Lys Asn Ser Leu Phe Glu 80 85 90	1840
TAT CAG AAG AAC AAT AAG GAT TCT CAT TCG TTG ACC ACT AAT ATA ATG Tyr Gln Lys Asn Asn Lys Asp Ser His Ser Leu Thr Thr Asn Ile Met 95 100 105 110	1888
GAA ATT TTG AGA GGC GAT TTT TCC TCA GCC AAT A GTAAGTATTAA Glu Ile Leu Arg Gly Asp Phe Ser Ser Ala Asn 115 120	1932
CATATTTACT TCTTGACTT TATAACAGAA ACAACAAAAA TCCTAAATAA ATATGATATC CGCTTATATC TATGACAATT TCATCCAAA GTACTTAGTG TAGAACACA TACCTTCATA ATATCCCTGA AAATTTAAG AGGGAGCTTT TGTTTTCGTT ATTTTTCAA AGTAAAAGAT GTAACTGAG ATTGTTAAG GTCACAAAAT AAGTCAGAAT TTTGGATTAA AACAAAGAATT TAAATGTGTT CTTTCAACA GTATATACTG AAAGTAGGAT GGGTCAGACT CTTTGAGTTG ATATTTTGT TTCTGCTTTG TAAAGGTGAA AACTGAGAGG TCAAGGAAC TGTCAAAGA CACAGAGCTG GGAATTCAAC TCCCAGACTC CACTGAGCTG ATTAGGTAGA TTTTAAATT TAAAATATAG GGTCAAGCTA CGTCATTCTC ACAGTCTACT CATTAGGGTT AGGAAACATT GCATTCACTC TGGGCATGGA CAGCGAGTCT AGGGAGTCCT CAGTTCTCA AGTTTGCTT TGCCTTTTA CACCTTCACA AACACTTGAC ATTTAAAATC AGTGATGCCA ACACAGCTG GCAAGTGAGT GATCCTGTTG ACCAAAAACA GCTTAGGAAC CATTCAAAT CTATAGAGTT AAAAAGAAAA GCTCATCAGT AAGAAAATCC AATATGTTCA AGTCCCTTGA TTAAGGATGT TATAAAATAA TTGAAATGCA ATCAAACAA CTATTTAAC TCCAAATTAC ACCTTTAAAA TTCCAAAGAA AGTTCTTCTT CTATATTCT TTGGGATTAC TAATTGCTAT TAGGACATCT TAACTGGCAT TCATGGAAGG CTGCAGGGCA TAACATTATC CAAAAGTCAA ATGCCCAATA	1992 2052 2112 2172 2232 2292 2352 2412 2472 2532 2592 2652 2712 2772 2832

GGTTTGAAAC TCACAGATTA AACTGTAACC AAAATAAAAT TAGGCATATT TACAAGCTAG	2892
TTTCTTCTT CTTTTTTCT CTTCTTTCT TTCTTTCTT CTTCTTTCT TTCTTTCTT	2952
CTTCTTTCT TTCTCCTTCC TTCTTTCTT CCTTTCTTT TTGCTGGCAA TTACAGACAA	3012
ATCACTCAGC AGCTACTTCA ATAACCATAT TTTCGATTTCA AG AC CGT GAT AAT	3065
Asn Arg Asp Asn	
125	
ACC TAC AAC CGA GTG TCA GAG GAT CTG AGA AGC AGA ATT GAA GTC CTG	3113
Thr Tyr Asn Arg Val Ser Glu Asp Leu Arg Ser Arg Ile Glu Val Leu	
130 135 140	
AAG CGC AAA GTC ATA GAA AAA GTA CAG CAT ATC CAG CTT CTG CAG AAA	3161
Lys Arg Lys Val Ile Glu Lys Val Gln His Ile Gln Leu Leu Gln Lys	
145 150 155	
AAT GTT AGA GCT CAG TTG GTT GAT ATG AAA CGA CTG GAG GTAAGTATGT	3210
Asn Val Arg Ala Gln Leu Val Asp Met Lys Arg Leu Glu	
160 165 170	
GGCTGTGGTC CCGAGTGTCC TTGTTTTGA GTAGAGGGAA AAGGAAGGCG ATAGTTATGC	3270
ACTGAGTGTCT TACTATATGC AGAGAAAAGT GTTATATCCA TCATCTACCT AAAAGTAGGT	3330
ATTATTTCC TCACTCCACA GTTGAAGAAA AAAAATTCA GAGATTTAA GTAAATTTCC	3390
CAACGTACAT AGATAGTAAT TCAAAGCAAT GTTCAGTCCC TGTCTATTCC AAGCCATTAC	3450
ATCACCACAC CTCTGAGCCC TCAGCCTGAG TTCACCAAGG ATCATTAAAT TAGCGTTCC	3510
TTTGAGAGGG AATAGCACCT TACTCTTGAT CCATTCTGAG GCTAAGATGA ATTAAACAGC	3570
ATCCATTGCT TATCCTGGCT AGCCCTGCAA TACCCAACAT CTCTTCACT GAGGGTGCTC	3630
GATAGGCAGA AAACAGAGAA TATTAAGTGG TAGGTCTCCG AGTCAAAAAA AATGAAACCA	3690
GTTTCCAGAA GGAAAATTAA CTACCAGGAA CTCATAGAC GTAGTTTATG TATTTGTATC	3750
TACATTTCT CTTTATTTT CTCCCCCTCTC TCTAG GTG GAC ATT GAT ATT AAG	3803
Val Asp Ile Asp Ile Lys	
175	

39

ATC CGA TCT TGT CGA GGG TCA TGC AGT AGG GCT TTA GCT CGT GAA GTA Ile Arg Ser Cys Arg Gly Ser Cys Ser Arg Ala Leu Ala Arg Glu Val 180 185 190	3851
GAT CTG AAG GAC TAT GAA GAT CAG CAG AAG CAA CTT GAA CAG GTC ATT Asp Leu Lys Asp Tyr Glu Asp Gln Gln Lys Gln Leu Glu Gln Val Ile 195 200 205	3899
GCC AAA GAC TTA CTT CCC TCT AGA GAT AGG CAA CAC TTA CCA CTG ATA Ala Lys Asp Leu Leu Pro Ser Arg Asp Arg Gln His Leu Pro Leu Ile 210 215 220	3947
AAA ATG AAA CCA GTT CCA GAC TTG GTT CCC GGA AAT TTT AAG AGC CAG Lys Met Lys Pro Val Pro Asp Leu Val Pro Gly Asn Phe Lys Ser Gln 225 230 235 240	3995
CTT CAG AAG GTA CCC CCA GAG TGG AAG GCA TTA ACA GAC ATG CCG CAG Leu Gln Lys Val Pro Pro Glu Trp Lys Ala Leu Thr Asp Met Pro Gln 245 250 255	4043
ATG AGA ATG GAG TTA GAG AGA CCT GGT GGA AAT GAG ATT ACT CGA GGA Met Arg Met Glu Leu Glu Arg Pro Gly Gly Asn Glu Ile Thr Arg Gly 260 265 270	4091
GGC TCC ACC TCT TAT GGA ACC GGA TCA GAG ACG GAA AGC CCC AGG AAC Gly Ser Thr Ser Tyr Gly Thr Gly Ser Glu Thr Glu Ser Pro Arg Asn 275 280 285	4139
CCT AGC AGT GCT GGA AGC TGG AAC TCT GGG AGC TCT GGA CCT GGA AGT Pro Ser Ser Ala Gly Ser Trp Asn Ser Gly Ser Ser Gly Pro Gly Ser 290 295 300	4187
ACT GGA AAC CGA AAC CCT GGG AGC TCT GGG ACT GGA GGG ACT GCA ACC Thr Gly Asn Arg Asn Pro Gly Ser Ser Gly Thr Gly Gly Thr Ala Thr 305 310 315 320	4235
TGG AAA CCT GGG AGC TCT GGA CCT GGA AGT GCT GGA AGC TGG AAC TCT Trp Lys Pro Gly Ser Ser Gly Pro Gly Ser Ala Gly Ser Trp Asn Ser 325 330 335	4283
GGG AGC TCT GGA ACT GGA AGT ACT GGA AAC CAA AAC CCT GGG AGC CCT Gly Ser Ser Gly Thr Gly Ser Thr Gly Asn Gln Asn Pro Gly Ser Pro 340 345 350	4331
AGA CCT GGT AGT ACC GGA ACC TGG AAT CCT GGC AGC TCT GAA CGC GGA Arg Pro Gly Ser Thr Gly Thr Trp Asn Pro Gly Ser Ser Glu Arg Gly 355 360 365	4379

AGT GCT GGG CAC TGG ACC TCT GAG AGC TCT GTA TCT GGT AGT ACT GGA Ser Ala Gly His Trp Thr Ser Glu Ser Ser Val Ser Gly Ser Thr Gly 370 375 380	4427
CAA TGG CAC TCT GAA TCT GGA AGT TTT AGG CCA GAT AGC CCA GGC TCT Gln Trp His Ser Glu Ser Gly Ser Phe Arg Pro Asp Ser Pro Gly Ser 385 390 395 400	4475
GGG AAC GCG AGG CCT AAC AAC CCA GAC TGG GGC ACA TTT GAA GAG GTG Gly Asn Ala Arg Pro Asn Asn Pro Asp Trp Gly Thr Phe Glu Glu Val 405 410 415	4523
TCA GGA AAT GTA AGT CCA GGG ACA AGG AGA GAG TAC CAC ACA GAA AAA Ser Gly Asn Val Ser Pro Gly Thr Arg Arg Glu Tyr His Thr Glu Lys 420 425 430	4571
CTG GTC ACT TCT AAA GGA GAT AAA GAG CTC AGG ACT GGT AAA GAG AAG Leu Val Thr Ser Lys Gly Asp Lys Glu Leu Arg Thr Gly Lys Glu Lys 435 440 445	4619
GTC ACC TCT GGT AGC ACA ACC ACC ACG CGT CGT TCA TGC TCT AAA ACC Val Thr Ser Gly Ser Thr Thr Thr Arg Arg Ser Cys Ser Lys Thr 450 455 460	4667
GTT ACT AAG ACT GTT ATT GGT CCT GAT GGT CAC AAA GAA GTT ACC AAA Val Thr Lys Thr Val Ile Gly Pro Asp Gly His Lys Glu Val Thr Lys 465 470 475 480	4715
GAA GTG GTG ACC TCC GAA GAT GGT TCT GAC TGT CCC GAG GCA ATG GAT Glu Val Val Thr Ser Glu Asp Gly Ser Asp Cys Pro Glu Ala Met Asp 485 490 495	4763
TTA GGC ACA TTG TCT GGC ATA GGT ACT CTG GAT GGG TTC CGC CAT AGG Leu Gly Thr Leu Ser Gly Ile Gly Thr Leu Asp Gly Phe Arg His Arg 500 505 510	4811
CAC CCT GAT GAA GCT GCC TTC TTC GAC ACT GCC TCA ACT GGA AAA ACA His Pro Asp Glu Ala Ala Phe Phe Asp Thr Ala Ser Thr Gly Lys Thr 515 520 525	4859
TTC CCA GGT TTC TTC TCA CCT ATG TTA GGA GAG TTT GTC AGT GAG ACT Phe Pro Gly Phe Phe Ser Pro Met Leu Gly Glu Phe Val Ser Glu Thr 530 535 540	4907

41

GAG TCT AGG GGC TCA GAA TCT GGC ATC TTC ACA AAT ACA AAG GAA TCC Glu Ser Arg Gly Ser Glu Ser Gly Ile Phe Thr Asn Thr Lys Glu Ser 545 550 555 560	4955
AGT TCT CAT CAC CCT GGG ATA GCT GAA TTC CCT TCC CGT GGT AAA TCT Ser Ser His His Pro Gly Ile Ala Glu Phe Pro Ser Arg Gly Lys Ser 565 570 575	5003
TCA AGT TAC AGC AAA CAA TTT ACT AGT AGC ACG AGT TAC AAC AGA GGA Ser Ser Tyr Ser Lys Gln Phe Thr Ser Ser Thr Ser Tyr Asn Arg Gly 580 585 590	5051
GAC TCC ACA TTT GAA AGC AAG AGC TAT AAA ATG GCA GAT GAG GCC GGA Asp Ser Thr Phe Glu Ser Lys Ser Tyr Lys Met Ala Asp Glu Ala Gly 595 600 605	5099
AGT GAA GCC GAT CAT GAA GGA ACA CAT AGC ACC AAG AGA GGC CAT GCT Ser Glu Ala Asp His Glu Gly Thr His Ser Thr Lys Arg Gly His Ala 610 615 620	5147
AAA TCT CGC CCT GTC AGA GGT ATC CAC ACT TCT CCT TTG GGG AAG CCT Lys Ser Arg Pro Val Arg Gly Ile His Thr Ser Pro Leu Gly Lys Pro 625 630 635 640	5195
TCC CTG TCC CCC TAGACTAAGT TAAATATTTC TGACAGTGT TCCCATGGCC Ser Leu Ser Pro 645	5247
CCTTGCATTT CCTTCTTAAC TCTCTGTTAC ACGTCATTGA AACTACACTT TTTTGGTCTG	5307
TTTTTGCT AGACTGTAAG TTCCCTGGGG GCAGGGCCTT TGTCTGTCTC ATCTCTGTAT	5367
TCCCAAATGC CTAACAGTAC AGAGCCATGA CTCATAAAAT ACATGTTAAA TGGATGAATG	5427
AATT CCTCTG AAACTCTATT TGAGCTTATT TAGTCAAATT CTTTCACTAT TCAAAGTGTG	5487
TGCTATTAGA ATTGTCACCC AACTGATTAA TCACATTTT AGTATGTGTC TCAGTTGACA	5547
TTTAGGTCAG GCTAAATACA AGTTGTGTTA GTATTAAGTG AGCTTAGCTA CCTGTACTGG	5607
TTACTTGCTA TTAGTTGTG CAAGTAAAT TCCAAATACA TTTGAGGAAA ATCCCCTTTG	5667
CAATTGTAG GTATAAATAA CCGCTTATTT GCATAAGTTC TATCCCACTG TAAGTGCATC	5727
CTTTCCCTAT GGAGGGAAGG AAAGGAGGAA GAAAGAAAGG AAGGGAAAGA AACAGTATT	5787
GCCTTATTAA ATCTGAGCCG TGCCATCTT TGAAAGTTA AATGAGAATA ACTTCTTCCA	5847

ACCAAGCTTAA TTTTTTTTTT AGACTGTGAT GATGTCTCC AAACACATCC TTCAGGTACC 5907

CAAAGTGGCA TTTTCAATAT CAAGCTATCC GGATCC 5943

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 644 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Met Phe Ser Met Arg Ile Val Cys Leu Val Leu Ser Val Val Gly Thr
1 5 10 15

Ala Trp Thr Ala Asp Ser Gly Glu Gly Asp Phe Leu Ala Glu Gly Gly
20 25 30

Gly Val Arg Gly Pro Arg Val Val Glu Arg His Gln Ser Ala Cys Lys
35 40 45

Asp Ser Asp Trp Pro Phe Cys Ser Asp Glu Asp Trp Asn Tyr Lys Cys
50 55 60

Pro Ser Gly Cys Arg Met Lys Gly Leu Ile Asp Glu Val Asn Gln Asp
65 70 75 80

Phe Thr Asn Arg Ile Asn Lys Leu Lys Asn Ser Leu Phe Glu Tyr Gln
85 90 95

Lys Asn Asn Lys Asp Ser His Ser Leu Thr Thr Asn Ile Met Glu Ile
100 105 110

Leu Arg Gly Asp Phe Ser Ser Ala Asn Asn Arg Asp Asn Thr Tyr Asn
115 120 125

Arg Val Ser Glu Asp Leu Arg Ser Arg Ile Glu Val Leu Lys Arg Lys
130 135 140

Val Ile Glu Lys Val Gln His Ile Gln Leu Leu Gln Lys Asn Val Arg
145 150 155 160

43

Ala Gln Leu Val Asp Met Lys Arg Leu Glu Val Asp Ile Asp Ile Lys
165 170 175

Ile Arg Ser Cys Arg Gly Ser Cys Ser Arg Ala Leu Ala Arg Glu Val
180 185 190

Asp Leu Lys Asp Tyr Glu Asp Gln Gln Lys Gln Leu Glu Gln Val Ile
195 200 205

Ala Lys Asp Leu Leu Pro Ser Arg Asp Arg Gln His Leu Pro Leu Ile
210 215 220

Lys Met Lys Pro Val Pro Asp Leu Val Pro Gly Asn Phe Lys Ser Gln
225 230 235 240

Leu Gln Lys Val Pro Pro Glu Trp Lys Ala Leu Thr Asp Met Pro Gln
245 250 255

Met Arg Met Glu Leu Glu Arg Pro Gly Gly Asn Glu Ile Thr Arg Gly
260 265 270

Gly Ser Thr Ser Tyr Gly Thr Gly Ser Glu Thr Glu Ser Pro Arg Asn
275 280 285

Pro Ser Ser Ala Gly Ser Trp Asn Ser Gly Ser Ser Gly Pro Gly Ser
290 295 300

Thr Gly Asn Arg Asn Pro Gly Ser Ser Gly Thr Gly Gly Thr Ala Thr
305 310 315 320

Trp Lys Pro Gly Ser Ser Gly Pro Gly Ser Ala Gly Ser Trp Asn Ser
325 330 335

Gly Ser Ser Gly Thr Gly Ser Thr Gly Asn Gln Asn Pro Gly Ser Pro
340 345 350

Arg Pro Gly Ser Thr Gly Thr Trp Asn Pro Gly Ser Ser Glu Arg Gly
355 360 365

Ser Ala Gly His Trp Thr Ser Glu Ser Ser Val Ser Gly Ser Thr Gly
370 375 380

Gln Trp His Ser Glu Ser Gly Ser Phe Arg Pro Asp Ser Pro Gly Ser
385 390 395 400

Gly Asn Ala Arg Pro Asn Asn Pro Asp Trp Gly Thr Phe Glu Glu Val
405 410 415

Ser Gly Asn Val Ser Pro Gly Thr Arg Arg Glu Tyr His Thr Glu Lys
420 425 430

Leu Val Thr Ser Lys Gly Asp Lys Glu Leu Arg Thr Gly Lys Glu Lys
435 440 445

Val Thr Ser Gly Ser Thr Thr Thr Arg Arg Ser Cys Ser Lys Thr
450 455 460

Val Thr Lys Thr Val Ile Gly Pro Asp Gly His Lys Glu Val Thr Lys
465 470 475 480

Glu Val Val Thr Ser Glu Asp Gly Ser Asp Cys Pro Glu Ala Met Asp
485 490 495

Leu Gly Thr Leu Ser Gly Ile Gly Thr Leu Asp Gly Phe Arg His Arg
500 505 510

His Pro Asp Glu Ala Ala Phe Phe Asp Thr Ala Ser Thr Gly Lys Thr
515 520 525

Phe Pro Gly Phe Phe Ser Pro Met Leu Gly Glu Phe Val Ser Glu Thr
530 535 540

Glu Ser Arg Gly Ser Glu Ser Gly Ile Phe Thr Asn Thr Lys Glu Ser
545 550 555 560

Ser Ser His His Pro Gly Ile Ala Glu Phe Pro Ser Arg Gly Lys Ser
565 570 575

Ser Ser Tyr Ser Lys Gln Phe Thr Ser Ser Thr Ser Tyr Asn Arg Gly
580 585 590

Asp Ser Thr Phe Glu Ser Lys Ser Tyr Lys Met Ala Asp Glu Ala Gly
595 600 605

Ser Glu Ala Asp His Glu Gly Thr His Ser Thr Lys Arg Gly His Ala
610 615 620

Lys Ser Arg Pro Val Arg Gly Ile His Thr Ser Pro Leu Gly Lys Pro
625 630 635 640

Ser Leu Ser Pro

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 8878 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(vii) IMMEDIATE SOURCE:

- (B) CLONE: human fibrinogen B-beta chain

(ix) FEATURE:

- (A) NAME/KEY: misc_RNA
- (B) LOCATION: 1..469

(ix) FEATURE:

- (A) NAME/KEY: exon
- (B) LOCATION: 470..583

(ix) FEATURE:

- (A) NAME/KEY: intron
- (B) LOCATION: 584..3257

(ix) FEATURE:

- (A) NAME/KEY: exon
- (B) LOCATION: 3258..3449

(ix) FEATURE:

- (A) NAME/KEY: intron
- (B) LOCATION: 3450..3938

(ix) FEATURE:

- (A) NAME/KEY: exon
- (B) LOCATION: 3939..4122

(ix) FEATURE:

- (A) NAME/KEY: intron
- (B) LOCATION: 4123..5042

(ix) FEATURE:

- (A) NAME/KEY: exon
- (B) LOCATION: 5043..5270

- (ix) FEATURE:
 - (A) NAME/KEY: intron
 - (B) LOCATION: 5271..5830

- (ix) FEATURE:
 - (A) NAME/KEY: exon
 - (B) LOCATION: 5831..5944

- (ix) FEATURE:
 - (A) NAME/KEY: intron
 - (B) LOCATION: 5945..6632

- (ix) FEATURE:
 - (A) NAME/KEY: exon
 - (B) LOCATION: 6633..6758

- (ix) FEATURE:
 - (A) NAME/KEY: intron
 - (B) LOCATION: 6759..6966

- (ix) FEATURE:
 - (A) NAME/KEY: exon
 - (B) LOCATION: 6967..7252

- (ix) FEATURE:
 - (A) NAME/KEY: intron
 - (B) LOCATION: 7253..7870

- (ix) FEATURE:
 - (A) NAME/KEY: exon
 - (B) LOCATION: 7871..8102

- (ix) FEATURE:
 - (A) NAME/KEY: 3'UTR
 - (B) LOCATION: 8103..8537

- (ix) FEATURE:
 - (A) NAME/KEY: misc_RNA
 - (B) LOCATION: 8538..8878

- (ix) FEATURE:
 - (A) NAME/KEY: CDS
 - (B) LOCATION: join(470..583, 3258..3449, 3939..4122, 5043..5270, 5831..5944, 6633..6758, 6967..7252, 7871..8102)

- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

GAATTCATGC CCCTTTGAA ATAGACTTAT GTCATTGTCA GAAAACATAA GCATTTATGG	60	
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CTTAGATTTG TTTTCTCAC A TATTCTTCG GAGCTTGTGT AGTTTCCACA TTAATTTACC	180	
AGAAACAAGA TACACACTCT CTTTGAGGAG TGCCCTAACT TCCCATCATT TTGTCCAATT	240	
AAATGAATTG AAGAAATT A ATGTTCTAA ACTAGACCAA CAAAGAATAA TAGTTGTATG	300	
ACAAGTAAAT AAGCTTGCT GGGAAAGATGT TGCTTAAATG ATAAAATGGT TCAGCCAACA	360	
AGTGAACCAA AAATTAATAA TTAACTAAGG AAAGGTAACC ATTTCTGAAG TCATTCTAG	420	
CAGAGGACTC AGATATATAT AGGATTGAAG ATCTCTAGT TAAGTCTAC ATG AAA	475	
Met Lys		
1		
AGG ATG GTT TCT TGG AGC TTC CAC AAA CTT AAA ACC ATG AAA CAT CTA	523	
Arg Met Val Ser Trp Ser Phe His Lys Leu Lys Thr Met Lys His Leu		
5	10	15
TTA TTG CTA CTA TTG TGT GTT TTT CTA GTT AAG TCC CAA GGT GTC AAC	571	
Leu Leu Leu Leu Leu Cys Val Phe Leu Val Lys Ser Gln Gly Val Asn		
20	25	30
GAC AAT GAG GAG GTGAATTTT TAAAGCATTA TTATATTATT AGTAGTATT A	623	
Asp Asn Glu Glu		
35		
TTAATATAAG ATGTAACATA ATCATATTAT GTGCTTATT TTAATGAAATT AGCATTGCTT	683	
ATAGTTATGA AATGGAATTG TTAACCTCTG ACTTATTGTA TTTAAAGAAT GTTTCATAGT	743	
ATTTCTATA TAAAAACAAA GTAATTCTT GTTTCTAGT TTATCACCTT TGTTTTCTTA	803	
AGATGAGGAT GGCTTAGCTA ATGTAAGATG TGTTTTCTC ACTTGCTATT CTGAGTACTG	863	
TGATTTTCAT TTACTTCTAG CAATACAGGA TTACAATTAA GAGGACAAGA TCTGAAAATC	923	
TCACAAACTA TAAAATAATA AAAGAGCAGA ATTTAAGAT AAAAGAAACT GGTGGTAGGT	983	
AGATTGTTCT TTGGTGAAGG AAGGTAATAT ATATTGTTAC TGAGATTACT ATTATATAAA	1043	
ATTATAACTA AGCCTAAAAG CAAAATACAT CAAGTGTAA T GATAGAAAAT GAAATATTGC	1103	

TTTTTCAGA TGAAAAGTTC AAATTAGAGT TAGTGTGTAT TGTTATTATT AATAGTTATG	1163
AAACACGGTT CAGTCTAATT TATTTATTTG TAGAACAGTT TGTCTCAAC TATTATTTT	1223
GCTGACTTAT TGCTGTTAAT TTGCAGTTAC TAAAATACA GAAATGCATT TAGGACAATG	1283
GATATTAAG AAATTTAAAT TTTATCATCA AACGTATCAT GGCAAATTT CTTACATATA	1343
GCATAGTATC ATTAAACTAG AAATAAGAAT ACACAATAAT ATTTAAATGA AGTGATTATC	1403
TTCGGATCAT TATTGAGTTT CAAGGGAAC TGAGTGTGT ACTTATCAGA CTCTACATGT	1463
AAGAACATAT AGTTAATCTG GTTGTGTGT TAAAACATA TGTTAATCT GGTTAAGTCT	1523
GGTTAATCAT ATTAGGTAAG AAAAATGTAA AGAATGTGTAGACGAAATT TTTGTAAAGT	1583
ACTCTGAAA GCACCTTCAC ATTTCTGCTT ATCAACTAAA CCTCACAGAG ATAGTTAAT	1643
AGTTAGGCT TAAAAATGGA TTTTGATTAT TCAACAAGTG GCCTTCATAA TTTCTTTAAG	1703
TGTTTTCTT TAAGTATATA CTTTCTTAA ATATTTTTA AAATTCCTT TTCTCTAGTA	1763
AAGCCAGACC ATCCATGCTA CCTCTCTAGT GGCACTCTGA AATAAAAAGA AAATAGTTT	1823
CTCTGTTATA ATTGTATTTG TAATAAGCAG ATGAATCACA TTTCTTTAAA TTTGTTTTAG	1883
AGAGGGTAAG CTCTGACTAG GACCATGACT TCAATGTGAA ATATGTATAT ATCCTCCGAA	1943
TCTTACATA TTAAGAATGT ATATAGTCAA CTGGTTAAC AGGAAAATCT GGAACAGCCT	2003
GGCTGGGTTT TAATCTTAGC ACCATCCTAC TAAATGTTAA ATAATATTAT AATCTAATGA	2063
ATAATGACA ATGCAATTCC AAATAGAGTT CATCTGATGA CTTCTAGACT CACAAAATTG	2123
CAAGAGAGCT CAGTTGTTGC TCAGTTGTTTC CAAATCATGT CGTTGTTAA TTTGTAATTA	2183
AGCTCCAAAG GATGTATAGC TACTGACAAA AAAAAAAATG AGAATGTAGT TAATCCAAAT	2243
CAAAACTTTC CTATTGCAAT GCGTATTTTC TGCTTCATTA TCCTTTAATA TAATATTITA	2303
AGTTAGCAAG TAATTTTAAT TACAATGCAC AAGCCTTGAG AATTATTTA AATATAAGAA	2363
AATCATAATG TTTGATAAAG AAATCATGTAGA AGAAATTCA AGATAATGGT TTAACAAATA	2423
ATTTTGTGA TAGAAGATAA GACTAAAAGT GAAATTGAA GTGGAGAGGA CACTTAAACT	2483
GTAGTACTTG TTATGTGTGA TTCCAGTAAA AATAGTAATG AGCACTTATT ATTGCCAAGT	2543

ACTGTTCTGA GGGTACCAT A TGCAATAAGT TATTTAATCC TTACAATAAT CTTGTAAGGC	2603
AGATTCAAAC TATCATTACA CTTATTTAC AGATGAGAAA ACTGGGGCAC AGATAAAGCA	2663
ACTTGCCCAA GGTCTCATAG CTGTAAGTC ACCCTACGGT CAAGACCTAC AAGTAGCCGA	2723
GCTCCAGAGT ACATTATGAG GGTCAAAGAT TGTCTTATTA CAAATAAATT CCAAGTAGAA	2783
TCAACCTTTA ATAAGTCTTT AATGTCCTT AAATATGTTT ATATAGGAGT CTAATCACCA	2843
ATTCACAAAA ATGAAAGTAG GGAAATGATT AACAAATAATC ATAGGAATCT AACAAATCCAA	2903
GTGGCTTGAG AATATTCACTT CTTCTTGACA GTATAGATT CTTACAATT CGTAAGTTCC	2963
AATGTATGTT TTAGGAATAT GAGGTCTTAA CTATTCTAA TCTGATACAG CTTTATCCTA	3023
AGGCCTCTCT TTAAAAACTA CACTGCATCA TAGCTTTTT GTGCAGTTGG TCTTTCTACT	3083
GTTACTGAAC AGTAAGCAAC CTACAGATT C ACTATCACCA ACCAGCCAGT TGATGGATCT	3143
TAAGCAAATT ATCAAGCTTG TGATAACCTA AATTATAAAA TGAGGGTGT GGAATAGTTA	3203
CATTCCAAAT CTTCTATAAC ACTCTGTATT ATATTCTGC CTCATTCTT GTAG GGT Gly	3260
 TTC TTC AGT GCC CGT GGT CAT CGA CCC CTT GAC AAG AAG AGA GAA GAG Phe Phe Ser Ala Arg Gly His Arg Pro Leu Asp Lys Lys Arg Glu Glu 40 45 50 55	3308
GCT CCC AGC CTG AGG CCT GCC CCA CCG CCC ATC AGT GGA GGT GGC TAT Ala Pro Ser Leu Arg Pro Ala Pro Pro Pro Ile Ser Gly Gly Gly Tyr 60 65 70	3356
CGG GCT CGT CCA GCC AAA GCA GCT GCC ACT CAA AAG AAA GTA GAA AGA Arg Ala Arg Pro Ala Lys Ala Ala Ala Thr Gln Lys Lys Val Glu Arg 75 80 85	3404
AAA GCC CCT GAT GCT GGA GGC TGT CTT CAC GCT GAC CCA GAC CTG Lys Ala Pro Asp Ala Gly Gly Cys Leu His Ala Asp Pro Asp Leu 90 95 100	3449
GTGGGTGCAC TGATGTTCT TGCAGTGGTG GCTCTCTCAT GCAGAGAAAG CCTGTAGTC	3509
TGGCAGTCTG CTAATGTTTC ACTGACCCAC ATTACCATCA CTGTTATTTT GTTGTTAT	3569

50

TTTGGAAATA AAATTCAAAA CATAAACATA TTGGGCCTTT GGTTTAGGCT TTCTTCTTG	3629
TTTCTTGG TCTGGGCCA AAATTCAAA TTAGGATATG TGGGTGCCAC CTTCCATT	3689
GTATTTGCC ACTGCCTTG TTTAGTTGGT AAAATTTCA TAGCCATT ATATTTTTC	3749
TGGGTAAGT AATATTTAA ATCTCTATGA GAGTATGATG ATGACTTCG AATTCTGGT	3809
CTTACAGAAA ACCAATAAT AAATTTAT GTTGGCTAAT CGTATCGCTG AATTTCCCTA	3869
TGTGCTATTT TAA AA ATGT CCATGACCCA AATCCTTCAT CTAATGCCCTG CTATTTCTT	3929
TGTTTTAG GGG GTG TTG TGT CCT ACA GGA TGT CAG TTG CAA GAG GCT Gly Val Leu Cys Pro Thr Gly Cys Gln Leu Gln Glu Ala 105 110 115	3977
TTG CTA CAA CAG GAA AGG CCA ATC AGA AAT AGT GTT GAT GAG TTA AAT Leu Leu Gln Gln Glu Arg Pro Ile Arg Asn Ser Val Asp Glu Leu Asn 120 125 130	4025
AAC AAT GTG GAA GCT GTT TCC CAG ACC TCC TCT TCT TCC TTT CAG TAC Asn Asn Val Glu Ala Val Ser Gln Thr Ser Ser Ser Phe Gln Tyr 135 140 145	4073
ATG TAT TTG CTG AAA GAC CTG TGG CAA AAG AGG CAG AAG CAA GTA AAA G Met Tyr Leu Leu Lys Asp Leu Trp Gln Lys Arg Gln Lys Gln Val Lys 150 155 160	4122
GTAGATATCC TTGTGCTTTC CATTGATTT TCAGCTATAA AATTGGAACC GTTAGACTGC	4182
CACGAGAATG CATGGTTGTG AGAAGATTAA CATTCTGGG TTAGTGAATA GCATTCTAC	4242
GCTTTGGGC ACCTTCCCT GCAACTTGCC AGATAAGCAC TATTCAGCTC TTATTCCCAG	4302
TCTGACATCA GCAAGTGTGA TTTCTATGA AAAATTCTAC TATGACTCCT TATTTAAGT	4362
ATACAAGAAA CTTGTGACTC AGAAGATAAT ATTTACAGAG TGGAAAAAAA CCCCTAGCAT	4422
TTATAGTTT AACATTTGAG GTTTGAATG AGAGAGTTAT CCATAATATA TTCAATTGTG	4482
TTGTGGATAA TGACACCTAA CCTGTGAATC TTGAGGTCAAG AATGTTGAGT GCTGTTGACT	4542
TGGTGGTCAG GAAACAGCTA GTGCGTGAGC CTGGCACAGG CATCTCAGTG AGTAGCATAAC	4602
CCACAGTTGG AAATTTTCA AAGAAATCAA AGGAATCATG ACATCTTATA AATTCAAGG	4662
TTCTGCTATA CTTATGTGAA ATGGATAAT AAATCAAGCA TATCCACTCT GTAAGATTGA	4722

ACTTCTCAGA TGGAAGACCC CAATACTGCT TTCTCCTCTT TTCCCTCACC AAAGAAATAA	4782
ACAAACCTATT TCATTTATTAA CTGGACACAA TCTTTAGCGT ATACCTATGG TAAATTACTA	4842
GTATGGTGGT TAGGATTAT GTTAATTTGT ATATGTCATG CGCCAAATCA TTTCCACTAA	4902
ATATGACTAT ATATCATAAC TGCTTGGTGA TAGCTCAGTG TTTAATAGTT TATTCTCAGA	4962
AAATCAAAAT TGTATAGTTA AATACATTAG TTTTATGAGG CAAAAATGCT AACTATTTCT	5022
ACATAATTC ATTTTTCCAG AT AAT GAA AAT GTA GTC AAT GAG TAC TCC Asp Asn Glu Asn Val Val Asn Glu Tyr Ser 165 170	5071
TCA GAA CTG GAA AAG CAC CAA TTA TAT ATA GAT GAG ACT GTG AAT AGC Ser Glu Leu Glu Lys His Gln Leu Tyr Ile Asp Glu Thr Val Asn Ser 175 180 185	5119
AAT ATC CCA ACT AAC CTT CGT GTG CTT CGT TCA ATC CTG GAA AAC CTG Asn Ile Pro Thr Asn Leu Arg Val Leu Arg Ser Ile Leu Glu Asn Leu 190 195 200 205	5167
AGA AGC AAA ATA CAA AAG TTA GAA TCT GAT GTC TCA GCT CAA ATG GAA Arg Ser Lys Ile Gln Lys Leu Glu Ser Asp Val Ser Ala Gln Met Glu 210 215 220	5215
TAT TGT CGC ACC CCA TGC ACT GTC AGT TGC AAT ATT CCT GTG GTG TCT Tyr Cys Arg Thr Pro Cys Thr Val Ser Cys Asn Ile Pro Val Val Ser 225 230 235	5263
GGC AAA G GTAACTGATT CATAAACATA TTTTAGAGA GTTCCAGAAG AACTCACACA Gly Lys	5320
CCAAAAATAA GAGAACACA ACAACACAA AAATGCTAAG TGGATTTCC CAACAGATCA	5380
TAATGACATT ACAGTACATC ATAAAATAT CCTTAGCCAG TTGTGTTTG GACTGGCTG	5440
GTGCATTTGC TGGTTTGAT GAGCAGGATG GGGCACAGGT AGTCCCAGGG GTGGCTGATG	5500
TGTGCATCTG CGTACTGGCT TGAACAGATG GCAGAACAC AGATAGATGT AGAAGTTCT	5560
CCATTTGTG TGTTCTGGGA GCTCATGGAT ATTCCAGGAC ACAAAAGGTG GAGAAGAGCT	5620
TTGTTCATCC TCTTAGCAGA TAAACGTCTT CAAACTGGG TTGGACTTAC TAAAGTAAAA	5680

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TGAAAATCTA ATATTTGTTA TATTATTTTC AAAGGTCTAT AATAACACAC TCCTTAGTAA	5740	
CTTATGTAAT GTTATTTAA AGAATTGGTG ACTAAATACA AAGTAATTAT GTCATAAACCC	5800	
CCTGAACATA ATGTTGTCTT ACATTTGCAG AA TGT GAG GAA ATT ATC AGG AAA	5853	
Glu Cys Glu Glu Ile Ile Arg Lys		
240	245	
GGA GGT GAA ACA TCT GAA ATG TAT CTC ATT CAA CCT GAC AGT TCT GTC	5901	
Gly Gly Glu Thr Ser Glu Met Tyr Leu Ile Gln Pro Asp Ser Ser Val		
250	255	260
AAA CCG TAT AGA GTA TAC TGT GAC ATG AAT ACA GAA AAT GGA G	5944	
Lys Pro Tyr Arg Val Tyr Cys Asp Met Asn Thr Glu Asn Gly		
265	270	275
GTAAGCTTTC GACAGTTGTT GACCTGTTGA TCTGTAATTA TTTGGATACC GTAAAATGCC	6004	
AGGAAACAAG GCCAGGTGTG GTGGCTCAT A CCTGTAATTC CAGCACCTG GGAGGCCAAA	6064	
GTGGGCTGAT AGCTTGAGCC TAGGAGTTG AACTAGCCT GGGCACATA ATGAGACCT	6124	
AACTCTACAA AAAAAAAA AATACCAAAA AAAAAAAA AATCAGCTGT GTTGGTAGTA	6184	
TGTGCCTGTA GTCCCAGCTA TCCAGGAGGC TGAGATGGGA GATCACCTGA GCCCACAAACC	6244	
TGGAGTCTTG ATCATGCTAC TGAACGTAG CCTGGGCAAC AGAGGATAGT GAGATCCTGT	6304	
CTCAAAAAAA AAAATTAAATT AAAAGCCAG GAAACAAGAC TTAGCTCTAA CATCTAACAT	6364	
AGCTGACAAA GGAGTAATT GATGTGGAAT TCAACCTGAT ATTTAAAGT TATAAAATAT	6424	
CTATAATTCA CAATTTGGGG TAAGATAAAG CACTTGCAGT TTCCAAAGAT TTTACAAGTT	6484	
TACCTCTCAT ATTTATTTCC TTATTGTGTC TATTTAGAG CACCAAATAT ATACTAAATG	6544	
GAATGGACAG GGGATTCAAGA TATTATTTTC AAAGTGACAT TATTTGCTGT TGGTTAATAT	6604	
ATGCTCTTT TGTTTCTGTC AACCAAAG GA TGG ACA GTG ATT CAG AAC CGT	6655	
Gly Trp Thr Val Ile Gln Asn Arg		
280	285	
CAA GAC GGT AGT GTT GAC TTT GGC AGG AAA TGG GAT CCA TAT AAA CAG	6703	
Gln Asp Gly Ser Val Asp Phe Gly Arg Lys Trp Asp Pro Tyr Lys Gln		
290	295	300

53

GGA TTT GGA AAT GTT GCA ACC AAC ACA GAT GGG AAG AAT TAC TGT GGC 6751
 Gly Phe Gly Asn Val Ala Thr Asn Thr Asp Gly Lys Asn Tyr Cys Gly
 305 310 315

CTA CCA G GTAACGAACA GGCATGCAAA ATAAAATCAT TCTATTTGAA ATGGGATTTT 6808
 Leu Pro

TTTAATTAA AAAACATTCA TTGTTGGAAG CCTGTTTAG GCAGTTAAGA GGAGTTTCCT 6868

GACAAAAATG TGGAAGCTAA AGATAAGGGA AGAAAGGCAG TTTTAGTTT CCCAAAATT 6928

TATTTTGTT GAGAGATTTT ATTTGTTTT TCTTTAG GT GAA TAT TGG CTT 6980
 Gly Glu Tyr Trp Leu
 320

GGA AAT GAT AAA ATT AGC CAG CTT ACC AGG ATG GGA CCC ACA GAA CTT 7028
 Gly Asn Asp Lys Ile Ser Gln Leu Thr Arg Met Gly Pro Thr Glu Leu
 325 330 335 340

TTG ATA GAA ATG GAG GAC TGG AAA GGA GAC AAA GTA AAG GCT CAC TAT 7076
 Leu Ile Glu Met Glu Asp Trp Lys Gly Asp Lys Val Lys Ala His Tyr
 345 350 355

GGA GGA TTC ACT GTA CAG AAT GAA GCC AAC AAA TAC CAG ATC TCA GTG 7124
 Gly Gly Phe Thr Val Gln Asn Glu Ala Asn Lys Tyr Gln Ile Ser Val
 360 365 370

AAC AAA TAC AGA GGA ACA GCC GGT AAT GCC CTC ATG GAT GGA GCA TCT 7172
 Asn Lys Tyr Arg Gly Thr Ala Gly Asn Ala Leu Met Asp Gly Ala Ser
 375 380 385

CAG CTG ATG GGA GAA AAC AGG ACC ATG ACC ATT CAC AAC GGC ATG TTC 7220
 Gln Leu Met Gly Glu Asn Arg Thr Met Thr Ile His Asn Gly Met Phe
 390 395 400

TTC AGC ACG TAT GAC AGA GAC AAT GAC GGC TG GTATGTGTGG 7262
 Phe Ser Thr Tyr Asp Arg Asp Asn Asp Gly Trp
 405 410 415

CACTCTTGC TCCTGCTTTA AAAATCACAC TAATATCATT ACTCAGAACATC ATTAACAATA 7322

TTTTTAATAG CTACCACTTC CTGGGCACCTT ACTGTCAGCC ACTGTCCTAA GCTCTTTATG 7382

CATCACTCGA AAGCATTCA ACTATAAGGT AGACATTCTT ATTCTCATT TACAGATGAG 7442

ATTTAGAGAG ATTACGTGAT TTGTCCAATG TCACACAACT ACCCAGAGAT AAAACTAGAA 7502

TTTGAGCACA GTTACTTTCT GAATAATGAG CATTAGATA AATACTATA TCTCTATATT	7562		
CTAAAGTGTG TGTGAAAAC TTCACTTTCA TTTCCAGGGT TCTCTGATAC TAAGGGTTGT	7622		
AAAAGCTATT ATTCCAGTAT AAAGTAACAA ACACAGTCCC TAGATGGATT GCCACAAAGG	7682		
CCCAGTTATC TCTCTTCTT GCTATAGGGC ACAGGAGGTC TTTGGTGTAT TAGTGTGACT	7742		
CTATGTATAG CACCCAAAGG AAAGACTACT GTGCACACGA GTGTAGCAGT CTTTTATGGG	7802		
TAATCTGCAA AACGTAACCTT GACCACCGTA GTTCTGTTTC TAATAACGCC AAACACATT	7862		
TCTTCAG G TTA ACA TCA GAT CCC AGA AAA CAG TGT TCT AAA GAA GAC	7910		
Leu Thr Ser Asp Pro Arg Lys Gln Cys Ser Lys Glu Asp			
420	425		
GGT GGT GGA TGG TGG TAT AAT AGA TGT CAT GCA GCC AAT CCA AAC GGC	7958		
Gly Gly Gly Trp Trp Tyr Asn Arg Cys His Ala Ala Asn Pro Asn Gly			
430	435	440	
AGA TAC TAC TGG GGT GGA CAG TAC ACC TGG GAC ATG GCA AAG CAT GGC	8006		
Arg Tyr Tyr Trp Gly Gly Gln Tyr Thr Trp Asp Met Ala Lys His Gly			
445	450	455	460
ACA GAT GAT GGT GTA GTA TGG ATG AAT TGG AAG GGG TCA TGG TAC TCA	8054		
Thr Asp Asp Gly Val Val Trp Met Asn Trp Lys Gly Ser Trp Tyr Ser			
465	470	475	
ATG AGG AAG ATG AGT ATG AAG ATC AGG CCC TTC TTC CCA CAG CAA TAGTCCCCAA			
8109			
Met Arg Lys Met Ser Met Lys Ile Arg Pro Phe Phe Pro Gln Gln			
480	485	490	
TACGTAGATT TTTGCTCTTC TGTATGTGAC AACATTTTG TACATTATGT TATTGGAATT	8169		
TTCTTTCATA CATTATATTC CTCTAAAACCTCTAAGCAGA CGTGAGTGTG ACTTTTGAA	8229		
AAAAGTATAG GATAAATTAC ATTAAAATAG CACATGATT TCTTTGTTT TCTTCATTTC	8289		
TCTTGCTCAC CCAAGAAGTA ACAAAAGTAT AGTTTGACA GAGTTGGTGT TCATAATTTC	8349		
AGTTCTAGTT GATTGCGAGA ATTTCAAT AAGGAAGAGG GGTCTTTAT CCTTGTGTA	8409		
GGAAAACCAT GACGGAAAGG AAAAAGTGT GTTAAAAGT CCACTTTAA AACTATATT	8469		
ATTTATGTAG GATCTGTCAA AGAAAACCTTC CAAAAAGATT TATTAATTAA ACCAGACTCT	8529		

GTTGCAATAA GTTAATGTTT TCTTGTGTTG TAATCCACAC ATTCAATGAG TTAGGCTTTG	8589
CACTTGTAAAG GAAGGAGAAG CGTTCACAAAC CTCAAATAGC TAATAAACCG GTCTTGAATA	8649
TTTGAAGATT TAAAATCTGA CTCTAGGACG GGCACGGTGG CTCACGACTA TAATCCCAAC	8709
ACTTTGGGAG GCTGAGGCAG GCGGTACAA GGTCAGGAGT TCAAGACCAG CCTGACCAAT	8769
ATGGTGAAAC CCCATCTCTA CTAAAAATAC AAAAATTAGC CAGGCGTGGT GGCAGGTGCC	8829
TGTAGGTCCC AGCTAGCCTG TGAGGTGGAG ATTGCATTGA GCCAAGATC	8878

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 491 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Met Lys Arg Met Val Ser Trp Ser Phe His Lys Leu Lys Thr Met Lys			
1	5	10	15
His Leu Leu Leu Leu Leu Cys Val Phe Leu Val Lys Ser Gln Gly			
20	25	30	
Val Asn Asp Asn Glu Glu Gly Phe Phe Ser Ala Arg Gly His Arg Pro			
35	40	45	
Leu Asp Lys Lys Arg Glu Glu Ala Pro Ser Leu Arg Pro Ala Pro Pro			
50	55	60	
Pro Ile Ser Gly Gly Tyr Arg Ala Arg Pro Ala Lys Ala Ala Ala			
65	70	75	80
Thr Gln Lys Lys Val Glu Arg Lys Ala Pro Asp Ala Gly Gly Cys Leu			
85	90	95	
His Ala Asp Pro Asp Leu Gly Val Leu Cys Pro Thr Gly Cys Gln Leu			
100	105	110	

56

Gln Glu Ala Leu Leu Gln Gln Glu Arg Pro Ile Arg Asn Ser Val Asp
115 120 125

Glu Leu Asn Asn Asn Val Glu Ala Val Ser Gln Thr Ser Ser Ser Ser
130 135 140

Phe Gln Tyr Met Tyr Leu Leu Lys Asp Leu Trp Gln Lys Arg Gln Lys
145 150 155 160

Gln Val Lys Asp Asn Glu Asn Val Val Asn Glu Tyr Ser Ser Glu Leu
165 170 175

Glu Lys His Gln Leu Tyr Ile Asp Glu Thr Val Asn Ser Asn Ile Pro
180 185 190

Thr Asn Leu Arg Val Leu Arg Ser Ile Leu Glu Asn Leu Arg Ser Lys
195 200 205

Ile Gln Lys Leu Glu Ser Asp Val Ser Ala Gln Met Glu Tyr Cys Arg
210 215 220

Thr Pro Cys Thr Val Ser Cys Asn Ile Pro Val Val Ser Gly Lys Glu
225 230 235 240

Cys Glu Glu Ile Ile Arg Lys Gly Gly Glu Thr Ser Glu Met Tyr Leu
245 250 255

Ile Gln Pro Asp Ser Ser Val Lys Pro Tyr Arg Val Tyr Cys Asp Met
260 265 270

Asn Thr Glu Asn Gly Gly Trp Thr Val Ile Gln Asn Arg Gln Asp Gly
275 280 285

Ser Val Asp Phe Gly Arg Lys Trp Asp Pro Tyr Lys Gln Gly Phe Gly
290 295 300

Asn Val Ala Thr Asn Thr Asp Gly Lys Asn Tyr Cys Gly Leu Pro Gly
305 310 315 320

Glu Tyr Trp Leu Gly Asn Asp Lys Ile Ser Gln Leu Thr Arg Met Gly
325 330 335

Pro Thr Glu Leu Leu Ile Glu Met Glu Asp Trp Lys Gly Asp Lys Val
340 345 350

Lys Ala His Tyr Gly Gly Phe Thr Val Gln Asn Glu Ala Asn Lys Tyr
355 360 365

Gln Ile Ser Val Asn Lys Tyr Arg Gly Thr Ala Gly Asn Ala Leu Met
370 375 380

Asp Gly Ala Ser Gln Leu Met Gly Glu Asn Arg Thr Met Thr Ile His
385 390 395 400

Asn Gly Met Phe Phe Ser Thr Tyr Asp Arg Asp Asn Asp Gly Trp Leu
405 410 415

Thr Ser Asp Pro Arg Lys Gln Cys Ser Lys Glu Asp Gly Gly Trp
420 425 430

Trp Tyr Asn Arg Cys His Ala Ala Asn Pro Asn Gly Arg Tyr Tyr Trp
435 440 445

Gly Gly Gln Tyr Thr Trp Asp Met Ala Lys His Gly Thr Asp Asp Gly
450 455 460

Val Val Trp Met Asn Trp Lys Gly Ser Trp Tyr Ser Met Arg Lys Met
465 470 475 480

Ser Met Lys Ile Arg Pro Phe Phe Pro Gln Gln
485 490

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 10564 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(vii) IMMEDIATE SOURCE:

- (B) CLONE: human fibrinogen gamma chain

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: join(1799..1876, 1973..2017, 2207..2390, 2510
..2603, 4211..4341, 4645..4778, 5758..5942, 7426
..7703, 9342..9571)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

CTACACACTT CTTGAAGGCA AAGGCAATGC TGAAGTCACC TTTCATGTTCA AAATCATATT	60
AAAAAGTTAG CAAGATGTAATTTACAGTGT ACTATGTAATTTCTTTGTGAA TGATCAATAA	120
TTACATATTTCATTATATA TATTTTAGTA GATAATATTATATACATTC AACATTCTAA	180
ATATAGAAAG TTTACAGAGAAAATAAAAGC CTTTTTTCC AATCCTGTCC TCCACCTCTG	240
CATCCCATTCTTACAG AGGCAACTGA TTCAAGTCAT TACATAGTTA TTGAGTGTAA	300
ACTACAACTA TGTTAAGTAC AGCTATATAT GTTAGATGCC GTAGCCACAG AAATCAGTTT	360
ACAATCTAAT GCAGTGGATA CAGCATGTAT ACATATAATA TAAGGTTGCT ACAAAATGCTA	420
TCTGAGGTAG AGCTGTTGA AAGAATACTA ATACTAAAT GTTTAATTCA ACTGACTTGA	480
TTGACAAC TGTTAAGTAC AGCTATATAT GTTAGATGCC GTAGCCACAG AAATCAGTTT	540
TGGTGGTATG GTGATATGAT TGACAATAAC TGCTAAGTCAGAGGGATA TATTAAGGAG	600
GAGAAGAAAA GCAACAAATC TGGTTTGAT GTGTTCACTT TGTTATAATT ATTGATTATT	660
TACTGAATAT GAATATTAT CTTGTTTTGAGTCATAAA ATATACCTT GTAAAGACAG	720
AATTAAAGTA TTGTTTACAGTGTGTTTCAACTG GAGGCATTTC TCCCACAAAC ATATTCATC	780
AAAACCTATA ATAAGCTTGG TTCCAGAGGA AGAAATGAGG GATAACCAAA AATAGAGACA	840
TTAATAATAG TGTAACGCC AGTGTAAAT CTCATAGGC AGTGTGACA GACATGTTT	900
CCCAACACA AGGATGCTGT AAGGGCCAAA CAGAAATGAT GGCCCTCCC CAGCACCTCA	960
TTTGCCCT TCCTTCAGCT ATGCCTCTAC TCTCCTTAG ATACAAGGGA GGTGGATTTT	1020
TCTCTCTCT GAGATAGCTT GATGGAACCA CAGGAACAAT GAAGTGGCT CCTGGCTCTT	1080
TTCTCTGTGG CAGATGGGGT GCCATGCCA CCTTCAGACA AAGGGAAAGAT TGAGCTAAA	1140
AGCTCCCTGA GAAGTGAGAG CCTATGAACA TGGTTGACAC AGAGGGACAG GAATGTATT	1200
CCAGGGTCAT TCATTCCCTGG GAATAGTGAA CTGGGACATG GGGGAAGTCAGTCA GTCTCCTCCT	1260
GCCACAGCCA CAGATTTAAATAATAATGT TAACTGATCC CTAGGCTAAA ATAATAGTGT	1320
TAACTGATCC CTAAGCTAAG AAAGTTCTTT TGGTAATTCA GGTGATGGCA GCAGGACCCA	1380

TCTTAAGGAT AGACTAGGTT TGCTTAGTTC GAGGTCATAT CTGTTGCTC TCAGCCATGT	1440
ACTGGAAGAA GTTGCATCAC ACAGCCTCCA GGACTGCCCT CCTCCTCAC A GCAATGGATA	1500
ATGCTTCACT AGCCTTGCA GATAATTTG GATCAGAGAA AAAACCTTGA GCTGGGCCAA	1560
AAAGGAGGAG CTTCAACCTG TGTGAAAAT CTGGGAACCT GACAGTATAG GTTGGGGGCC	1620
AGGATGAGGA AAAAGGAACG GGAAAGACCT GCCCACCCCT CTGGTAAGGA GGCCCCGTGA	1680
TCAGCTCCAG CCATTTGCAG TCCTGGCTAT CCCAGGAGCT TACATAAAGG GACAATTGGA	1740
GCCTGAGAGG TGACAGTGCT GACACTACAA GGCTCGGAGC TCCGGGCACT CAGACATC	1798
ATG AGT TGG TCC TTG CAC CCC CGG AAT TTA ATT CTC TAC TTC TAT GCT Met Ser Trp Ser Leu His Pro Arg Asn Leu Ile Leu Tyr Phe Tyr Ala	1846
1 5 10 15	
CTT TTA TTT CTC TCT TCA ACA TGT GTA GCA GTAAAGTGTGC TCTTCACAAA Leu Leu Phe Leu Ser Ser Thr Cys Val Ala	1896
20 25	
ACGTTGTTTA AAATGGAAAG CTGGAAAATA AAACAGATAA TAAACTAGTG AAATTTCGT	1956
ATTTTTCTC TTTTAG TAT GTT GCT ACC AGA GAC AAC TGC TGC ATC TTA Tyr Val Ala Thr Arg Asp Asn Cys Cys Ile Leu	2005
30 35	
GAT GAA AGA TTC GTAAAGTAGTT TTTATGTTTC TCCCTTG TGTAACCTGG Asp Glu Arg Phe	2057
40	
AGAGGGGCAG AGGAATAGAA ATAATCCCT CATAAATATC ATCTGGCACT TGTAACCTTT	2117
TAAAAACATA GTCTAGGTTT TACCTATTT TCTTAATAGA TTTTAAGAGT AGCATCTGTC	2177
TACATTTTA ATCACTGTTA TATTTCAAG GGT AGT TAT TGT CCA ACT ACC TGT Gly Ser Tyr Cys Pro Thr Thr Cys	2230
45	
GGC ATT GCA GAT TTC CTG TCT ACT TAT CAA ACC AAA GTA GAC AAG GAT Gly Ile Ala Asp Phe Leu Ser Thr Tyr Gln Thr Lys Val Asp Lys Asp	2278
50 55 60 65	

60

CTA CAG TCT TTG GAA GAC ATC TTA CAT CAA GTT GAA AAC AAA ACA TCA
 Leu Gln Ser Leu Glu Asp Ile Leu His Gln Val Glu Asn Lys Thr Ser 2326
 70 75 80

GAA GTC AAA CAG CTG ATA AAA GCA ATC CAA CTC ACT TAT AAT CCT GAT
 Glu Val Lys Gln Leu Ile Lys Ala Ile Gln Leu Thr Tyr Asn Pro Asp 2374
 85 90 95

GAA TCA TCA AAA CCA A GTGAGAAAAT AAAGACTACT GACCAAAAAA 2420
 Glu Ser Ser Lys Pro
 100

TAATAATAAT AATCTGTGAA GTTCTTTGC TGTTGTTTA GTTGTCTAT TTGCTTAAGG 2480
 ATTTTATGT CTCTGATCCT ATATTACAG AT ATG ATA GAC GCT GCT ACT TTG
 Asn Met Ile Asp Ala Ala Thr Leu 2532
 105 110

AAG TCC AGG ATA ATG TTA GAA GAA ATT ATG AAA TAT GAA GCA TCG ATT 2580
 Lys Ser Arg Ile Met Leu Glu Glu Ile Met Lys Tyr Glu Ala Ser Ile
 115 120 125

TTA ACA CAT GAC TCA AGT ATT CG GTAAGGATT TTGTTTAAT TTGCTCTGCA 2633
 Leu Thr His Asp Ser Ser Ile Arg
 130

AGACTGATTT AGTTTTATT TAATATTCTA TACTTGAGTG AAAGTAATT TTAATGTGTT 2693
 TTCCCCATT ATAATATCCC AGTGACATTA TGCTGATTA TGTTGAGCAT AGTAGAGATA 2753
 GAAGTTTTA GTGCAATATA AATTATACTG GGTTATAATT GCTTATTAAT AATCACATTG 2813
 AAGAAAGATG TTCTAGATGT CTTCAAATGC TAGTTGACC ATATTATCA AAAATTTTT 2873
 CCCCATCCCC CATTATCTT ACAACATAAA ATCAATCTCA TAGGAATTG GGTGTTGAAA 2933
 ATAAAATCCT CTTTATAAAA ATGCTGACAA ATTGGTGGTT AAAAAAATTA GCAAGCAGAG 2993
 GCATAGTAAG GATTTGGCT CCTAAAGTAA ATTATATTGA ATGTGGAGCA GGAAGAAACA 3053
 TGTCTTGAGA GACTAAGTGT GGCAAATATT GCAAAGCTCA TATTGATCAT TGCGAGATGA 3113
 ACCTGCATAG TCTCTCCCT TCATTTGGAA GTGAATGTCT CTGTTAAAGC TTCTCAGGGA 3173
 CTCATAAACT TTCTGAACAT AAGGTCTCAG ATACAGTTT AATATTTTC CCCAATTTTT 3233
 TTTTCTGAAT TTTTCTCAA GCAGCTTGAG AAATTGAGAT AAATAGTAGC TAGGGAGAAG 3293

TGGCCCCAGGA AAGATTTCTC CTCTTTTGC TATCAGAGGG CCCTTGTAT TATTGTTATT	3353
ATTATTACTT GCATTATTAT TGTCCATCAT TGAAGTTGAA GGAGGTTATT GTACAGAAAT	3413
TGCCTAAGAC AAGGTAGAGG GAAAACGTGG ACAAAATAGTT TGTCTACCTC TTTTACTTC	3473
AAAGAAAGAA CGGTTTATGC ATTGTAGACA GTTTCTATC ATTTTTGGAT ATTTGCAAGC	3533
CACCCGTAA GTAACTACAA AAGGAGGGTT TTTACTTCCC CCAGTCCATT CCCAAAGCTA	3593
TGTAACCAGA AGCATTAAAG AAGAAAGGGG AAGTATCTGT TGTTTTATT TACATACAAT	3653
AACGTTCCAG ATCATGTCCC TGTGTAAGTT ATATTTAGA TTGAAGCTTA TATGTATAGC	3713
CTCAGTAGAT CCACAAGTGA AAGGTATACT CCTTCAGCAC ATGTGAATT CTGAAC TGAG	3773
CTTTCTGC TTCTAAAGCA TCAGGGGGTG TTCCTATTAA CCAGTCTCGC CACTCTTGCA	3833
GGTTGCTATC TGCTGTCCCT TATGCATAAA GTAAAAAGCA AAATGTCAAT GACATTTGCT	3893
TATTGACAAG GACTTTGTTA TTTGTGTTGG GAGTTGAGAC AATATGCCCTT ATTCTAAGTA	3953
AAAAGATTCA GGTCCACATT GTATTCTGT TTTAATTGAT TTTTGATTT GTTTTCTTT	4013
TTCAAAAAGT TTATAATT TT AATTCACTGTT AATTAGTAA TATAATT TT CATTCTC	4073
AAGAATGGAA TAATTATCA GAAAGCACTT CTTAAGAAAA TACTTAGCAG TTTCAAAGA	4133
AAATATAAAA TTACTCTTCT GAAAGGAATA CTTATTTTG TCTTCTTATT TTGTTATCT	4193
TATGTTCTG TTTGTAG A TAT TTG CAG GAA ATA TAT AAT TCA AAT AAT CAA Tyr Leu Glu Glu Ile Tyr Asn Ser Asn Asn Glu 135 140 145	4244
AAG ATT GTT AAC CTG AAA GAG AAG GTA GCC CAG CTT GAA GCA CAG TGC Lys Ile Val Asn Leu Lys Glu Val Ala Glu Leu Glu Ala Glu Cys 150 155 160	4292
CAG GAA CCT TGC AAA GAC ACG GTG CAA ATC CAT GAT ATC ACT GGG AAA G Glu Pro Cys Lys Asp Thr Val Glu Ile His Asp Ile Thr Gly Lys 165 170 175	4341
GTAACTGATG AAGGTTATAT TGGGATTAGG TTCATCAAAG TAAGTAATGT AAAGGAGAAA	4401
GTATGACTG GAAAGTATAG GAATAGTTA GAAAGTGGCT ACCCATTAAAG TCTAAGAATT	4461

TCAGTTGTCT AGACCTTCT TGAATAGCTA AAAAAACAG TTTAAAAGGA ATGCTGATGT	4521
GAAAAGTAAG AAAATTATTC TTGGAAAATG AATAGTTAC TACATGTTAA AAGCTATTT	4581
TCAAGGCTGG CACAGTCTTA CCTGCATTC AAACCACAGT AAAAGTCGAT TCTCCTCTC	4641
TAG AT TGT CAA GAC ATT GCC AAT AAG GGA GCT AAA CAG AGC GGG CTT Asp Cys Glu Asp Ile Ala Asn Lys Gly Ala Lys Glu Ser Gly Leu	4688
180 185 190	
TAC TTT ATT AAA CCT CTG AAA GCT AAC CAG CAA TTC TTA GTC TAC TGT Tyr Phe Ile Lys Pro Leu Lys Ala Asn Glu Glu Phe Leu Val Tyr Cys	4736
195 200 205	
GAA ATC GAT GGG TCT GGA AAT GGA TGG ACT GTG TTT CAG AAG Glu Ile Asp Gly Ser Gly Asn Glu Trp Thr Val Phe Glu Lys	4778
210 215 220	
GTAATTTTTT CCCCCACCATG TGTATTTAAT AAATTCCTAC ATTGTTCTG CCATATGGCA	4838
GATACTTTTC TAAGCACCTT GTGAACCGTA GCTCATTAA TCCTTGCAAT AGCCCTAAGA	4898
GGAAGGTACT TCTGTTACTC CTATTTACAG AAAAGGAAAC TGAGGCACAC AAGGTTAAAT	4958
AACTTGCCCCA AGACCACATA ACTAATAAGC AACAGAGTCAC GCATTGAAC CTAGGCAGTA	5018
TAGTTTCAGA GTTTGTGACT TGACTCTATA TTGTAUTGGC ACTGACTTTG TAGATTCTAG	5078
GTGGCACATA ATCATAGTAC CACAGTGACA AATAAAAAGA AGGAAACTCT TTTGTCAGGT	5138
AGGTCAAGAC CTGAGGTTTC CCATCACAAAG ATGAGGAAGC CCAACACCAC CCCCCACAC	5198
CCCAACCACCA TCACCACCCCT TTCAACACACC AGAGGATACA CTTGGGCTGC TCCAAGACAA	5258
GGAACCTGTG TTGCATCTGC CACTTGCTGA TACCCACTAG GAATCTTGGC TCCTTTACTT	5318
TCTGTTTACCC TCCCCACCACT GTTATAACTG TTTCTACAGG GGGCGCTCAG AGGGAATGAA	5378
TGGTGGAAAGC ATTAGTTGCC AGACACCGAT TGAGCAATGG GTTCCATCAT AAGTGTAAAGA	5438
ATCAGTAATA TCCAGCTAGA GTTCTGAAGT CGTCTAGGTG TCTTTTAAT ATTACCACTC	5498
ATTAGAATT TATGATGTGC CAGAAACCCCT CTTAAGTATT TCTCTTATAT TCTCTCTCAT	5558
GATCCTTGCA GCAACCCCTAA GAAGTAACCA TCATTTTCC TATTTGATAC ATGAGGAAAC	5618
TGAGGTAGCT TGGCCAAGAT CACTTAGTTG GGAGTTGATA GAACCAGTGC TCTGTATTTT	5678

TGACAAAATG TTGACAGCAT TCTCTTACA TGCATTGATA GTCTATTTTC TCCTTTGCT	5738		
CTTGCAGATG TGTAATTAG AGA CTT GAT GGC AGT GTA GAT TTC AAG AAA AAC	5790		
Arg Leu Asp Gly Ser Val Asp Phe Lys Lys Asn			
225	230		
TGG ATT CAA TAT AAA GAA GGA TTT GGA CAT CTG TCT CCT ACT GGC ACA	5838		
Trp Ile Gln Tyr Lys Glu Gly Phe Gly His Leu Ser Pro Thr Gly Thr			
235	240	245	
ACA GAA TTT TGG CTG GGA AAT GAG AAG ATT CAT TTG ATA AGC ACA CAG	5886		
Thr Glu Phe Trp Leu Gly Asn Glu Lys Ile His Leu Ile Ser Thr Gln			
250	255	260	265
TCT GCC ATC CCA TAT GCA TTA AGA GTG GAA CTG GAA GAC TGG AAT GGC	5934		
Ser Ala Ile Pro Tyr Ala Leu Arg Val Glu Leu Glu Asp Trp Asn Gly			
270	275	280	
AGA ACC AG GTACTGTTT GAAATGACTT CCAACTTTT ATTGTAAAGA	5982		
Arg Thr Ser			
TTGCCTGGAA TGTGCACTT CCAACTATCA ATAGACAATG GCAAATGCAG CCTGACAAAT	6042		
GCAACAGCA CATCCAGCCA CCATTTCTC CAGGAGTCTG TTTGGTTCTT GGGCAATCCA	6102		
AAAAGGTAAA TTCTATTCAG GATGAATCTA AGTGTATTGG TACAATCTAA TTACCCCTGGA	6162		
ACCATTCAAGA GTAATAGCTA ATTACTGAAC TTTAATCAG TCCCAGGAAT TGAGCATAAA	6222		
ATTATAATT TATCTAGTCT AAATTACTAT TTCATGAAGC AGGTATTATT ATTAATCCCA	6282		
TTTTATAGAT TAACTTGCTC AAAGTCACAT TGCTGATAAG TGGTAGAGGT AGAATTCAAGA	6342		
CTCAAGTAGT TAACTTTAG AGCCTGTCCT CTTAACAACT ATCCTGGTTG AAAAGCAAAT	6402		
ACAGCCTCTT CAGACTTCTC AGTGCCTTGA TGGCCATTAA TTCTGTCAA TCATGAGCTA	6462		
CCCTAAAAGT AAACCAGCTA GCTCTTTGA TGATCTAGAG GCTTCTTTT GCTTGAGATA	6522		
TTTGAAGGTT TTAAGCATTG TTACCTAATT AAAATGCAGA AAAATATCCA ACCCTTTGT	6582		
TATGTTTAAG GAATAGTGAA ATATATTGTC TTCAACACAA TGGACTTTT TTTATTGCTT	6642		
GGTTGGTTT TAATCCAGAA AGTGCTATAG TCAGTAGACC TTCTTCTAGG AAAGGACCTT	6702		

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CCATTTCCA GCCACTGGAG ATTAGAAAAT AAGCTAAATA TTTCTGGAA ATTTCTGTC	6762
ATTCATTAAG GCCCATCCTT TCCCCACTC TATAGAAGTG TTGTCACCT GCACAATTT	6822
TTCCAGGAAA GAATCTCTCT AACTCCTTCA GCTCACATGC TTTGGACCAC ACAGGGAAAGA	6882
CTTTGATTGT GTAATGCCCT CAGAAGCTCT CCTTCTTGCC ACTACCACAC TGATTTGAGG	6942
AAGAAAATCC CTTAGCACC TAACCCTTCA GGTGCTATGA GTGGCTAATG GAACTGTACC	7002
TCCTTCAAGT TTTGTGCAAT ATTAAGGGT CACTCACTGT CAGATACTT CTGTGATCTA	7062
TGATAATGTG TGTGCAACAC ATAACATTTC AATAAAAGTA GAAAATATGA AATTAGAGTC	7122
ATCTACACAT CTGGATTGTA TCTTAGAATG AAACAAGCAA AAAAGCATCC AAGTGAGTGC	7182
AATTATTAGT TTTCAGAGAT GCTCAAAGG CTTCTAGGCC CATCCGGGA AGTGTAAATG	7242
AGCTGTGGAC TGGTCACAT ATCTATTGCC TCTTGCCAGA TTTGCAAAAA ACTTCACTCA	7302
ATGAGCAAAT TTCAGCCTTA AGAAACAAAG TCAAAAATTC CAAGGAAGCA TCCTACGAAA	7362
GAGGGAACTT CTGAGATCCC TGAGGAGGGT CAGCATGTGA TGGTTGTATT TCCTTCTTCT	7422
CAG T ACT GCA GAC TAT GCC ATG TTC AAG GTG GGA CCT GAA GCT GAC Thr Ala Asp Tyr Ala Met Phe Lys Val Gly Pro Glu Ala Asp	7468
285 290 295	
AAG TAC CGC CTA ACA TAT GCC TAC TTC GCT GGT GGG GAT GCT GGA GAT Lys Tyr Arg Leu Thr Tyr Ala Tyr Phe Ala Gly Gly Asp Ala Gly Asp	7516
300 305 310	
GCC TTT GAT GGC TTT GAT TTT GGC GAT GAT CCT AGT GAC AAG TTT TTC Ala Phe Asp Gly Phe Asp Phe Gly Asp Asp Pro Ser Asp Lys Phe Phe	7564
315 320 325 330	
ACA TCC CAT AAT GGC ATG CAG TTC AGT ACC TGG GAC AAT GAC AAT GAT Thr Ser His Asn Gly Met Gln Phe Ser Thr Trp Asp Asn Asp Asn Asp	7612
335 340 345	
AAG TTT GAA GGC AAC TGT GCT GAA CAG GAT GGA TCT GGT TGG TGG ATG Lys Phe Glu Gly Asn Cys Ala Glu Gln Asp Gly Ser Gly Trp Trp Met	7660
350 355 360	
AAC AAG TGT CAC GCT GGC CAT CTC AAT GGA GTT TAT TAC CAA G Asn Lys Cys His Ala Gly His Leu Asn Gly Val Tyr Tyr Gln	7703
365 370 375	

GTATGTTTC CTTCTTAGA TTCCAAGTTA ATGTATAGTG TATACTATTT TCATAAAAAA	7763
TAATAAAATAG ATATGAAGAA ATGAAGAATA ATTTATAAAG ATAGTAGGGA TTTTATCATG	7823
TTCTTATTT CAACTAAGTT CTTGAAACT GGAAGTGGAT AATACCAAGT TCATGCCTAA	7883
AATTAGCCCT TCTAAAGAAA TCCACCTGCT GCAAAATATC CAGTAGTTG GCATTATATG	7943
TGAAACTATC ACCATCATAG CTGGCACTGT GGGTTGTGGG ATCTCCTTTA GACATACAAC	8003
ATAAAATGATC TGGATGGATT AACATTACTA CATGGATGCT TGTTGACACA TTAACCTGGC	8063
TTCCCATGAG CTTTGTGTCA GATACACGCA GTGAACAGGT GTTTGGAGGA ACAGAATAAA	8123
GAGAAGGCAA GCACTGGTAA GGGCAGGGGT TTGTGAAAGC TTGAGAGAAG AGACCAGTCT	8183
GAGGACAGTA GACACTTATT TTAGGATGGG GGTTGGATGA GGAGGCTATA GTTTGCTATA	8243
AGCTTGAAT GGTTTGAAC ACTGGTTCA CTCACCTACC CAGCAGTTAT GTGTGGGAA	8303
GCCTTACCGA TGCTAAAGGA TCCATGTTAC AATAATGGCA TTATTGGAA ATCCCAGTGG	8363
TATTCCATGA ATAAAACAC TATGAAGATA ATCCCACCTCA ACAGACTCTC CGTTGGAGAA	8423
GGACAGCAAC ACCACCCCTGG GAAAGCCAAA CAGTCAGACC AGACCTGTT AGCATCAGTA	8483
GGACTTCCCT ACCATATCTG CTGGGTAGAT GAGTGAAACC AGTGTCCAA ACCACTCCGG	8543
GCTTGTAGCA AACCATAGTC TCCTCATCTA CCAAGATGAG CAACCTTACC TCCTGATGTC	8603
CTAGCCAATC ACCAACTAGG AAACTTGCA CAGTTATTT AAAGTAACAG TTTGATTTTC	8663
ACAATATTT TAAATTGGAG AACATAACT TATCTTGCA CTCACAAACC ACATAATGAG	8723
AAGAAAATCT AAGGGAAAAT GCTTGATCTG TGTGACCCGG GGCGCCATGC CAGAGCTGTA	8783
GTTCATGCCA GTGTTGTGCT CTGACAAGCC TTTTACAGAA TTACATGAGA TCTGCTTCCC	8843
TAGGACAAGG AGAAGGCAA TCAACAGAGG CTGCACTTTA AAATGGAGAC ATAAAATAAC	8903
ATGCCAGAAC CATTCCCTAA AGCTCCTCAA TCAACCAACA AAATTGTGCT TTCAAATAAC	8963
CTGAGTTGAC CTCATCAGGA ATTTGTGGC TCCTTCTCTT CTAACCTGCC TGAAGAAAGA	9023
TGGTCCACAG CAGCTGAGTC CGGGATGGAT AAGCTTAGGG ACAGAGGCCA ATTAGGGAAC	9083

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TTTGGGTTTC TAGCCCTACT AGTAGTGAAT AAATTAAAG TGTGGATGTG ACTATGAGTC 9143
 ACAGCACAGA TGTTGTTAA TAATATGTTT ATTTTATAAA TTGATATTTT AGGAATCTT 9203
 GGAGATATTT TCAGTTAGCA GATAACTA TAAATTTAT GTAACTGGCA ATGCACTTCG 9263
 TAATAGACAG CTCTTCATAG ACTTGCAGAG GTAAAAAGAT TCCAGAATAA TGATATGTAC 9323
 ATCTACGACT TGTTTTAG GT GGC ACT TAC TCA AAA GCA TCT ACT CCT AAT 9373
 Gly Gly Thr Tyr Ser Lys Ala Ser Thr Pro Asn
 380 385

GGT TAT GAT AAT GGC ATT ATT TGG GCC ACT TGG AAA ACC CGG TGG TAT 9421
 Gly Tyr Asp Asn Gly Ile Ile Trp Ala Thr Trp Lys Thr Arg Trp Tyr
 390 395 400

TCC ATG AAG AAA ACC ACT ATG AAG ATA ATC CCA TTC AAC AGA CTC ACA 9469
 Ser Met Lys Lys Thr Thr Met Lys Ile Ile Pro Phe Asn Arg Leu Thr
 405 410 415

ATT GGA GAA GGA CAG CAA CAC CAC CTG GGG GGA GCC AAA CAG GTC AGA 9517
 Ile Gly Glu Gly Gln Gln His His Leu Gly Gly Ala Lys Gln Val Arg
 420 425 430 435

CCA GAG CAC CCT GCG GAA ACA GAA TAT GAC TCA CTT TAC CCT GAG GAT 9565
 Pro Glu His Pro Ala Glu Thr Glu Tyr Asp Ser Leu Tyr Pro Glu Asp
 440 445 450

GAT TTG TAGAAAATTA ACTGCTAACT TCTATTGACC CACAAAGTTT CAGAAATTCT 9621
 Asp Leu

CTGAAAGTTT CTTCTTTTT TCTCTTACTA TATTTATTGA TTTCAAGTCT TCTATTAAGG 9681
 ACATTTAGCC TTCAATGGAA ATTTAAACTC ATTTAGGACT GTATTTCAA ATTACTGATA 9741
 TCAGAGTTAT TTAAAAATTG TTTATTTGAG GAGATAACAT TTCAACTTG TTCTAAATA 9801
 TATAATAATA AAATGATTGA CTTTATTGCA ATTTTATGA CCACTTGTCA TTTATTTGT 9861
 CTTCGTAAAT TATTTTCATT ATATCAAATA TTTTAGTATG TACTTAATAA AATAGGAGAA 9921
 CATTTTAGAG TTTCAAATTC CCAGGTATTT TCCTTGTAA TTACCCCTAA ATCATTCTA 9981
 TTTAATTCTT CTTTTAAAT GGAGAAAATT ATGTCTTTT AATATGGTTT TTGTTTTGTT 10041
 ATATATTCAC AGGCTGGAGA CGTTAAAAG ACCGTTCAA AAGAGATTAA CTTTTTTAAA 10101

GGACTTTATC TGAACAGAGA GATATAATAT TTTCTTATT GGACAATGGA CTTGCAAAGC	10161
TTCACCTCAT TTTAAGAGCA AAAGACCCCA TGTTGAAAAC TCCATAACAG TTTTATGCTG	10221
ATGATAATTT ATCTACATGC ATTTCAATAA ACCTTTGTT TCCTAAGACT AGATACATGG	10281
TACCTTTATT GACCATTAAA AAACCACAC TTTTGCCAA TTTACCAATT ACAATTGGGC	10341
AACCATCAGT AGTAATTGAG TCCTCATTTT ATGCTAAATG TTATGCCTAA CTCTTGGGA	10401
GTTACAAAGG AAATAGCAAT TATGGCTTTT GCCCTCTAGG AGATACAGGA CAAATACAGG	10461
AAAATACAGC AACCCAAACT GACAATACTC TATACAAGAA CATAATCACT AAGCAGGAGT	10521
CACAGCCACA CAACCAAGAT GCATAGTATC CAAAGTGCAG CTG	10564

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 453 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Met Ser Trp Ser Leu His Pro Arg Asn Leu Ile Leu Tyr Phe Tyr Ala			
1	5	10	15
Leu Leu Phe Leu Ser Ser Thr Cys Val Ala Tyr Val Ala Thr Arg Asp			
20	25	30	
Asn Cys Cys Ile Leu Asp Glu Arg Phe Gly Ser Tyr Cys Pro Thr Thr			
35	40	45	
Cys Gly Ile Ala Asp Phe Leu Ser Thr Tyr Gln Thr Lys Val Asp Lys			
50	55	60	
Asp Leu Gln Ser Leu Glu Asp Ile Leu His Gln Val Glu Asn Lys Thr			
65	70	75	80
Ser Glu Val Lys Gln Leu Ile Lys Ala Ile Gln Leu Thr Tyr Asn Pro			
85	90	95	

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Asp Glu Ser Ser Lys Pro Asn Met Ile Asp Ala Ala Thr Leu Lys Ser
 100 105 110

Arg Ile Met Leu Glu Glu Ile Met Lys Tyr Glu Ala Ser Ile Leu Thr
 115 120 125

His Asp Ser Ser Ile Arg Tyr Leu Gln Glu Ile Tyr Asn Ser Asn Asn
 130 135 140

Gln Lys Ile Val Asn Leu Lys Glu Lys Val Ala Gln Leu Glu Ala Gln
 145 150 155 160

Cys Gln Glu Pro Cys Lys Asp Thr Val Gln Ile His Asp Ile Thr Gly
 165 170 175

Lys Asp Cys Gln Asp Ile Ala Asn Lys Gly Ala Lys Gln Ser Gly Leu
 180 185 190

Tyr Phe Ile Lys Pro Leu Lys Ala Asn Gln Gln Phe Leu Val Tyr Cys
 195 200 205

Glu Ile Asp Gly Ser Gly Asn Gly Trp Thr Val Phe Gln Lys Arg Leu
 210 215 220

Asp Gly Ser Val Asp Phe Lys Lys Asn Trp Ile Gln Tyr Lys Glu Gly
 225 230 235 240

Phe Gly His Leu Ser Pro Thr Gly Thr Thr Glu Phe Trp Leu Gly Asn
 245 250 255

Glu Lys Ile His Leu Ile Ser Thr Gln Ser Ala Ile Pro Tyr Ala Leu
 260 265 270

Arg Val Glu Leu Glu Asp Trp Asn Gly Arg Thr Ser Thr Ala Asp Tyr
 275 280 285

Ala Met Phe Lys Val Gly Pro Glu Ala Asp Lys Tyr Arg Leu Thr Tyr
 290 295 300

Ala Tyr Phe Ala Gly Gly Asp Ala Gly Asp Ala Phe Asp Gly Phe Asp
 305 310 315 320

Phe Gly Asp Asp Pro Ser Asp Lys Phe Phe Thr Ser His Asn Gly Met
 325 330 335

Gln Phe Ser Thr Trp Asp Asn Asp Asn Asp Lys Phe Glu Gly Asn Cys
 340 345 350

Ala Glu Gln Asp Gly Ser Gly Trp Trp Met Asn Lys Cys His Ala Gly
 355 360 365

His Leu Asn Gly Val Tyr Tyr Gln Gly Gly Thr Tyr Ser Lys Ala Ser
 370 375 380

Thr Pro Asn Gly Tyr Asp Asn Gly Ile Ile Trp Ala Thr Trp Lys Thr
 385 390 395 400

Arg Trp Tyr Ser Met Lys Lys Thr Thr Met Lys Ile Ile Pro Phe Asn
 405 410 415

Arg Leu Thr Ile Gly Glu Gln Gln His His Leu Gly Gly Ala Lys
 420 425 430

Gln Val Arg Pro Glu His Pro Ala Glu Thr Glu Tyr Asp Ser Leu Tyr
 435 440 445

Pro Glu Asp Asp Leu
 450

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 10807 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(vii) IMMEDIATE SOURCE:

- (B) CLONE: ovine beta-lactoglobulin

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

ACCGGTGTCG ACCTGCAGGT CAACGGATCT CTGTGTCTGT TTTCATGTTA GTACCACACT	60
GTGGTGGTGG CTGTAGCTTT CAGCTACAGT CTGAAGTCAT AAAGCCTGGT ACCTCCAGCT	120
CTGTTCTCTC TCAAGATTGT GTTCTGCTGT TTGGGTCTTT AGTGTCTCCA CACAATTTTT	180
AGAATTGTTT GTTCTAGTTC TGTAAAAAT GATGCTGGTA TTTTGATAAG GATTGCATTG	240
AATCTGTAAA GCTACAGATA TAGTCATTGG GTAGTACAGT CACTTAACA ATATTAACTC	300

70

TTCACATCTG TGAGCATGAT ATATTTCCC CCTCTATATC ATCTTCAATT CCTCCTATCA 360
 GTTCTTTCA TTGCAGTTT CTGAGTACAG GTCTTACACC TCCTGGTTA GAGTCATTCC 420
 TCAGTATTT ATTCTTTGA TACAATTGTG AATGAGGTA 480
 GATAGCTCAT TGTTAGTGTAT TATATAGAAA AGCAACAGAT TTCTATGTAT TAATTTGTA 540
 TCCTGCAACA GATTCTATG TATTAATTGT 600
 TAGCTTTTG GTGACATCTT GAGGATTTTC TGAAGAAAAT GGCATGGTAT GGTAGGACAA 660
 GGTGTCATGT CATCTGCAAA CAGTGGCAGT TTTCTTCTT CCCTTCAAC CTGGATTCT 720
 TTGATTTCTT TCTGTCTGAG TACGACTAGG ATTCCCAATA CTATACCGAA TAAAAGTGGC 780
 AAGAGTGGAC ATCCTTGTCT TATTTTCTG ACCTTAGAGG AAATGCTTTC AGTTTTTCAC 840
 CATTAATTAT AATGTTACT GTGGGCTTGT CATATGTGGC CTTCAATTATA TGGAGGTCTA 900
 TTCCCTCTAT ACCCACCTG TTGAGAGTTT TTATCATAAA AGTATGTTGA ATTTGTCAA 960
 AAGTTTTCC TGCATCTATT GAGATGATT TTACTCTTCA ATTCAATTAT GATTTTTATT 1020
 CTTCATTTTG TTAATGATT CCATTCTTCA ATTTGTTAAC GTGGTATATC ACATTGATTG 1080
 ATTTGTGGAT ACCTTTGTAT CCCTGGATA AACCTCACTT GATCATGAGC TTTCAATGTA 1140
 TTTTGAATT CACTTGCTA ATATTCTGTT GGGTATTTT GCATCTCTAT TCATCAATGTA 1200
 TATTGGCCTA AGAAAGGTTT TGTCTGGTT TAGTATCAGG GTGATGCTGG CCTCATAGAG 1260
 AGAGTTAGA AGCATTCTT CCTCTTTGAT TTTCGGAAT AGTTGAGTA GGATAGGTAT 1320
 TAACTCTTCT TTAAATGTTT GGGGACTTCC CTGGTGAGCC GGTGGTTGAG AATCCGCCCTC 1380
 AGGGATGTGG GTTTGATCCC TGGTCAGGGA ACCATTAATA AGATCCCACA TGCTGCAGGC 1440
 AACAAAGCCCC CAAGCTGCAA CCACTGAGCT GCAACCGCTG CAGTGCCAC AGGCCACGAC 1500
 CAGAGAAAGC CCACATACAG CAGGGAAGAC CCAGCACAAC CGGAAAAAGG AGTTTGGTGG 1560
 AATACAGCTG TGAAGCCGTC TGGTCTGGA CTCTGCTTG AGGGAATTAA 1620
 TTGATTCAAT TTCATTACTG GTAACTGGTC TGTTCATATT TTCTATTCT TCCGGGTTCA 1680
 GTCTTGGGAG ATTGTACATG CCTAGGAATG TGTCCGTTTC TTCTAGGTTG TCCATTAT 1740

TGGACATGCA TGGGAGCACA CAGCACCGAC CAGCGAGACT CATGCTGGCT TCCTGGGCC	1800
AGGCTGGGC CCCAAGCAGC ATGGCATCCT AGAGTGTGTG AAAGCCCCT GACCCCTGCC	1860
AGCCCCACAA TTTCATTCTG AGAAGTGATT CCTTGCTTCT GCACTTACAG GCCCAGGATC	1920
TGACCTGCTT CTGAGGGAGCA GGGGTTTGG CAGGACGGGG AGATGCTGAG AGCCGACGGG	1980
GGTCCAGGTC CCCTCCCAGG CCCCCCTGTC TGGGGCAGCC CTTGGGAAAG ATTGCCAG	2040
TCTCCCTCCT ACAGTGGTCA GTCCCAGCTG CCCCAGGCCA GAGCTGCTT ATTTCGTCT	2100
CTCTCTCTGG ATGGTATTCT CTGGAAGCTG AAGGTTCTG AAGTTATGAA TAGCTTGCC	2160
CTGAAGGGCA TGGTTTGTGG TCACGGTTCA CAGGAACCTG GGAGACCCCTG CAGCTCAGAC	2220
GTCCCGAGAT TGGTGGCACC CAGATTCCT AAGCTCGCTG GGGAACAGGG CGCTTGTTC	2280
TCCCTGGCTG ACCTCCCTCC TCCCTGCATC ACCCAGTTCT GAAAGCAGAG CGGTGCTGGG	2340
GTCACAGCCT CTGCGATCTA ACGCCGGTGT CCAAACCAAC CGTGCTGGTG TTCGGGGGGC	2400
TACCTATGGG GAAGGGCTTC TCACTGCAGT GGTGCCCCC GTCCCCCTG AGATCAGAAC	2460
TCCCAGTCCG GACGTCAAAC AGGCCGAGCT CCCTCCAGAG GCTCCAGGGA GGGATCCTTG	2520
CCCCCCCCGCT GCTGCCTCCA GCTCCTGGTG CCGCACCCCTT GAGCCTGATC TTGTAGACGC	2580
CTCAGTCTAG TCTCTGCCTC CGTGTTCACA CGCCTTCTCC CCATGTCCCC TCCGTGTCCC	2640
CGTTTTCTCT CACAAGGACA CCGGACATTA GATTAGCCCC TGTTCCAGCC TCACCTGAAC	2700
AGCTCACATC TGTAAAGACC TAGATTCAA ACAAGATTCC AACCTGAAGT TCCCAGTGG	2760
TGTGAGTTCT GGGGCGACAT CCTTCAACCC CATCACAGCT TGCAAGTTCAT CGCAAAACAT	2820
GGAACCTGGG GTTTATCGTA AAACCCAGGT TCTTCATGAA AACTGAGCT TCGAGGCTTG	2880
TTGCAAGAAT TAAAGGTGCT AATACAGATC AGGGCAAGGA CTGAAGCTGG CTAAGCCTCC	2940
TCTTTCATC ACAGGAAAGG GGGGCCTGGG GGCAGCTGGA GGTCTGCTCC CGTGAGTGAG	3000
CTCTTTCCTG CTACAGTCAC CAACAGTCTC TCTGGGAAGG AAACCAAGGG CCAGAGAGCA	3060
AGCCGGAGCT AGTTAGGAG ACCCCTGAAC CTCCACCCAA GATGCTGACC AGCCAGCGGG	3120

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CCCCCTGGAA AGACCCCTACA GTTCAGGGGG GAAGAGGGGC TGACCCGCCA GGTCCCTGCT	3180
ATCAGGAGAC ATCCCCGCTA TCAGGAGATT CCCCCACCTT GCTCCCGTTC CCCTATCCCA	3240
ATACGCCAC CCCACCCCTG TGATGAGCAG TTTAGTCACT TAGAATGTCA ACTGAAGGCT	3300
TTTGCATCCC CTTTGCCAGA GGCACAAGGC ACCCACAGCC TGCTGGGTAC CGACGCCAT	3360
GTGGATTCA G CAGGAGGCC TGTCCTGCAC CCTCCCTGCT CGGGCCCCCT CTGTGCTCAG	3420
CAACACACCC AGCACCAAGCA TTCCCGCTGC TCCTGAGGTC TGCAAGGCAGC TCGCTGTAGC	3480
CTGAGCGGTG TGGAGGGAAAG TGTCCTGGGA GATTTAAAAT GTGAGAGGCG GGAGGTGGGA	3540
GGTTGGGCC C TGTGGGCCTG CCCATCCCAC GTGCCTGCAT TAGCCCCAGT GCTGCTCAGC	3600
CGTGCCCCCG CCGCAGGGGT CAGGTCACTT TCCCCTGCCTG GGGTTATTAT GACTCTTGTC	3660
ATTGCCATTG CCATTTTG C TACCCCTAACT GGGCAGCAGG TGCTTGAGA GCCCTCGATA	3720
CCGACCAGGT CCTCCCTCGG AGCTCGACCT GAACCCCATG TCACCCCTGC CCCAGCCTGC	3780
AGAGGGTGGG TGACTGCAGA GATCCCTTCA CCCAAGGCCA CGGTACATG GTTGGAGGA	3840
GCTGGTGCC C AAGGCAGAGG CCACCCCTCCA GGACACACCT GTCCCCAGTG CTGGCTCTGA	3900
CCTGTCTTG TCTAAGAGGC TGACCCCGGA AGTGTTCCTG GCACTGGCAG CCAGCCTGGA	3960
CCCAGAGTCC AGACACCCAC CTGTGCCCCC GCTTCTGGGG TCTACCAGGA ACCGTCTAGG	4020
CCCAGAGGGG ACTTCCTGCT TGGCCTTGGGA TGGAAGAAGG CCTCCTATTG TCCTCGTAGA	4080
GGAAGCCACC CCGGGGGCCTG AGGATGAGCC AAGTGGGATT CCGGGAACCG CGTGGCTGGG	4140
GGCCCAGCCC GGGCTGGCTG GCCTGCATGC CTCCGTATA AGGCCCCAAG CCTGCTGTCT	4200
CAGCCCTCCA CTCCCTGCAG AGCTCAGAACG CACGACCCCA GGGATATCCC TGCAAGCCATG	4260
AAGTGCCTCC TGCTTGCCCT GGGCCTGGCC CTCGCCTGTG GCGTCCAGGC CATCATCGTC	4320
ACCCAGACCA TGAAAGGCCT GGACATCCAG AAGGTTCGAG GGTTGGCCGG GTGGGTGAGT	4380
TGCAGGGCGG GCAGGGGAGC TGGGCCTCAG AGAGCCAAGA GAGGCTGTGA CGTTGGGTTTC	4440
CCATCAGTCA GCTAGGGCCA CCTGACAAAT CCCCCTGGG GCAGCTCAA CCAGGGCTTC	4500
ACTGTCTTGC ATTCTGGAGG CTGGAAGCCC AAGATCCAGG TGTTGGCAGG GCTGGCTTCT	4560

CCTGCGGCCG CTCTCTGGGG AGCAGACGGC CGTCTTCTCC AGTCCTCTGC GCGCCCTGAT	4620
TTCCCTCTTCC TGTGAGGCCA CCAGGCCTGC TGGAAACACG CCTGCCTGCG CAGCTTCACA	4680
CGACCTTTGT CATCTCTTA AAGGCCATGT CTCCAGAGTC ATGTGTTGAA GTTCTGGGGG	4740
TTAGTGGGAC ACAGTTCAGC CCCTAAAAGA GTCTCTCTGC CCCTCAAATT TTCCCCACCT	4800
CCAGGCCATGT CTCCCCAAGA TCCAAATGTT GCTACATGTG GGGGGGCTCA TCTGGGTCCC	4860
TCTTGGGTT CAGTGTGAGT CTGGGGAGAG CATTCCCCAG GGTGCAGAGT TGGGGGGAGT	4920
ATCTCAGGGC TGCCCAGGCC GGGGTGGGAC AGAGAGCCCA CTGTGGGCT GGGGGCCCT	4980
TCCCACCCCC AGAGTGCAAC TCAAGGTCCC TCTCCAGGTG GCGGGGACTT GGCACTCCTT	5040
GGCTATGGCG GCCAGCGACA TCTCCCTGCT GGATGCCAG AGTCCCCCCC TGAGAGTGT	5100
CGTGGAGGAG CTGAAGCCCA CCCCCGAGGG CAACCTGGAG ATCCTGCTGC AGAAATGGTG	5160
GGCGTCTCTC CCCAACATGG AACCCCCACT CCCCAGGGCT GTGGACCCCC CGGGGGGTGG	5220
GGTGCAGGAG GGACCAGGGC CCCAGGGCTG GGGAAAGAGGG CTCAGAGTTT ACTGGTACCC	5280
GGCGCTCCAC CCAAGGCTGC CCACCCAGGG CTTTTTTTTT TTTAAACTT TTATTAATT	5340
GATGCITCAG AACATCATCA AACAAATGAA CATAAAACAT TCATTTTGT TTACTTGAA	5400
GGGGAGATAA AATCCTCTGA AGTGGAAATG CATAGCAAAG ATACATACAA TGAGGCAGGT	5460
ATTCTGAATT CCCTGTTAGT CTGAGGATTA CAAGTGTATT TGAGCAACAG AGAGACATT	5520
TCATCATTTC TAGTCTGAAC ACCTCAGTAT CTAATGAA CAAGAAGTCC TGGAAACGAA	5580
GCAGTGTGGG GATAGGCCCG TGTGAAGGCT GCTGGGAGGC AGCAGACCTG GGTCTCGGG	5640
CTCAAGCAGT TCCCGCTACC AGCCCTGTCC ACCTCAGACG GGGGTCAAGGG TGCAAGGAGAG	5700
AGCTGGATGG GTGTGGGGGC AGAGATGGGG ACCTGAACCC CAGGGCTGCC TTTTGGGGGT	5760
GCCTGTGGTC AAGGCTCTCC CTGACCTTTT CTCTCTGGCT TCATCTGACT TCTCCTGGCC	5820
CATCCACCCG GTCCCCGTG GCCTGAGGTG ACAGTGAGTG CGCCGAGGCT AGTTGGCCAG	5880
CTGGCTCCTA TGCCCAGGCC ACCCCCCCTCC AGCCCTCCTG GGCCAGCTTC TGCCCCCTGGC	5940

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CCTCAGTTCA	TCCTGATGAA	AATGGTCCAT	GCCAATGGCT	CAGAAAGCAG	CTGTCTTC	6000
GGGAGAACGG	CGAGTGTGCT	CAGAAGAAGA	TTATTGCAGA	AAAACCAAG	ATCCCTGCGG	6060
TGTTCAAGAT	CGATGGTGAG	TCCGGGTCCC	TGGGGGACAC	CCACCACCCC	CGCCCCCGGG	6120
GAATGTGGAC	AGGTTCAAGGG	GGCTGGCGTC	GGGCCCTGGG	ATGCTAAGGG	ACTGGTGGTG	6180
ATGAAGACAC	TGCCTTGACA	CCTGCTTCAC	TTGCCTCCCC	TGCCACCTGC	CCGGGGCCTT	6240
GGGGCGGTGG	CCATGGGCAG	GTCCCGGCTG	GCAGGGCTAAC	CCACCAGGGT	GACACCCGAG	6300
CTCTCTTGC	TGGGGGGCGG	GCAGGGCTCT	GGGCCCTCAG	GCTGAGCTCA	GGAGGTACCT	6360
GTGCCCTCCC	AGGGGTAACC	GAGAGCCGTT	GCCCACCTCA	GGGGCCCAGG	TGCCACCGA	6420
CCCCAGCCCC	CTCCACAGCT	CCTTCATCTC	CTGGAGACAA	ACTCTGTCCG	CCCTCGCTCA	6480
TTCACTTGTT	CGTCCTAAAT	CCGAGATGAT	AAAGCTTCGA	GGGGGGGTTG	GGGTTCCATC	6540
AGGGCTGCC	TTCCGCCGGG	CAGCCTGGGC	CACATCTGCC	CTTGGCCCCC	TCAGGACTCA	6600
CTCTGACTGG	AGGCCCTGCA	CTGACTGACG	CCAGGGTGCC	CAGCCAGGG	TCTCTGGCGC	6660
CATCCAGCTG	CACTGGGTTT	GGGTGCTGGT	CCTGCCCTCA	AGCTGCCCGG	ACACCACAGG	6720
CAGCCGGGGC	TGCCCACTGG	CCTCGGTCA	GGTGAGCCCC	AGCTGCCCTC	GCTCAGGGCT	6780
TGCCCGACA	ATGACCCAT	CCTCAGGACG	CACCCCCCTT	CCCTTGCTGG	GCAGTGTCCA	6840
GCCCCACCCG	AGATCGGGGG	AAGCCCTATT	TCTTGACAAC	TCCAGTCCCT	GGGGGAGGGG	6900
GCTCAGACT	GAGTGGTGAG	TGTTCCCAAG	TCCAGGAGGT	GGTGGAGGGT	CCTGGCGGAT	6960
CCAGAGTTGA	CAGTGAGGGC	TTCCCTGGCC	CCATGCGCCT	GGCAGTGGCA	GCAGGGAAGA	7020
GGAAGCACCA	TTTCAGGGGT	GGGGGATGCC	AGAGGCGCTC	CCCACCCCGT	CTTCGCCGGG	7080
TGGTGACCCC	GGGGGAGCCC	CGCTGGTCGT	GGAGGGTGCT	GGGGGCTGAC	TAGCAACCCC	7140
TCCCCCCCCG	TTGGAACCTCA	CTTTCTCCC	GTCTTGACCG	CGTCCAGCCT	TGAATGAGAA	7200
CAAAGTCCTT	GTGCTGGACA	CCGACTACAA	AAAGTACCTG	CTCTTCTGCA	TGGAAAACAG	7260
TGCTGAGCCC	GAGCAAAGCC	TGGCCTGCCA	GTGCCTGGGT	GGGTGCCAAC	CCTGGCTGCC	7320
CAGGGAGACC	AGCTGCGTGG	TCCTTGCTGC	AACAGGGGGT	GGGGGGTGGG	AGCTTGATCC	7380

CCAGGGAGGAG GAGGGGTGGG GGGTCCCTGA GTCCCGCCAG GAGAGAGTGG TCGCATACCG	7440
GGAGCCAGTC TGCTGTGGC CTGTGGGTGG CTGGGGACGG GGGCCAGACA CACAGGCCGG	7500
GAGACGGGTG GGCTGCAGAA CTGTGACTGG TGTGACCGTC GCGATGGGC CGGTGGTCAC	7560
TGAATCTAAC AGCCTTTGTT ACCGGGGAGT TTCAATTATT TCCCAAAATA AGAACTCAGG	7620
TACAAAGCCA TCTTTCAACT ATCACATCCT GAAAACAAAT GGCAAGGTGAC ATTTTCTGTG	7680
CCGTAGCAGT CCCACTGGC ATTTTCAGGG CCCCTGTGCC AGGGGGCGC GGGCATCGC	7740
GAGTGGAGGC TCCTGGCTGT GTCAGCCGGC CCAGGGGGAG GAAGGGACCC GGACAGCCAG	7800
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GTGGACAAACG AGGCCCTGGA GAAATTGAC AAAGCCCTCA AGGCCCTGCC CATGCACATC	8040
CGGCTTGCCT TCAACCCGAC CCAGCTGGAG GGTGAGCACC CAGGCCCCGC CCTTCCCCAG	8100
GGCAGGAGCC ACCCGGCCCC GGGACGACCT CCTCCCA1GG TGACCCCCAG CTCCCCAGGC	8160
CTCCCAAGGAG GAAGGGGTGG GGTGCAGCAC CCCGTGGGGG CCCCCCTCCCC ACCCCCTGCC	8220
AGGCCTCTCT TCCCAGGGTG TCCAGTCCCA TCCTGACCCC CCCATGACTC TCCCTCCCCC	8280
ACAGGGCAGT GCCACGTCTA GGTGAGCCCC TGCCGGTGCC TCTGGGTAA GCTGCCTGCC	8340
CTGCCCAACG TCCTGGGCAC ACACATGGGG TAGGGGGTCT TGGTGGGGCC TGGGACCCCA	8400
CATCAGGCC C TGGGGTCCCC CCTGTGAGAA TGGCTGGAAG CTGGGGTCCC TCCTGGCGAC	8460
TGCAGAGCTG GCTGGCCCGCG TGCCACTCTT GTGGGTGACC TGTGTCTGG CCTCACACAC	8520
TGACCTCCTC CAGCTCCTTC CAGCAGAGCT AAGGCTAAGT GAGCCAGAAT GGTACCTAAG	8580
GGGAGGGCTAG CGGTCTTCT CCCGAGGGAGG GGCTGTCTG GAACCACCAAG CCATGGAGAG	8640
GCTGGCAAGG GTCTGGCAGG TGCCCCAGGA ATCACAGGGG GGCCCCATGT CCATTTCAAGG	8700
GCCCCGGGAGC CTTGGACTCC TCTGGGGACA GACGACGTCA CCACCGCCCC CCCCCCATCA	8760

GGGGGACTAG AAGGGACCAAG GACTGCAGTC ACCCTTCCTG GGACCCAGGC CCCTCCAGGC	8820
CCCTCCTGGG GCTCCTGCTC TGGCAGCTT CTCCCTCACC AATAAAGGCA TAAACCTGTG	8880
CTCTCCCTTC TGAGTCTTTG CTGGACGACG GGCAGGGGGT GGAGAAAGTGG TGGGGAGGGA	8940
GTCTGGCTCA GAGGATGACA GCAGGGCTGG GATCCAGGGC GTCTGCATCA CAGTCTTGTG	9000
ACAACCTGGGG GCCCACACAC ATCACTGCGG CTCTTGAAA CTTTCAGGAA CCAGGGAGGG	9060
ACTCGGCAGA GACATCTGCC AGTTCACTTG GAGTGTTAG TCAACACCCA AACTCGACAA	9120
AGGACAGAAA GTGGAAAATG GCTGTCTCTT AGTCTAATAA ATATTGATAT GAAACTCAAG	9180
TTGCTCATGG ATCAATATGC CTTTATGATC CAGCCAGCCA CTACTGTCGT ATCAACTCAT	9240
GTACCCAAAC GCACTGATCT GTCTGGCTAA TGATGAGAGA TTCCCAGTAG AGAGCTGGCA	9300
AGAGGTACA GTGAGAACTG TCTGCACACA CAGCAGAGTC CACCAGTCAT CCTAAGGAGA	9360
TCAGTCCTGG TGTTCATTGG AGGACTGATG TTGAAGCTGA AACTCCAATG CTTTGGCCAC	9420
CTGATGTGAA GAGCTGACTC ATTTGAAAAG ACCCTGATGC TGGGAAAGAT TGAGGGCAGG	9480
AGGAGAAGGG GACGACAGAG GATGAGATGG TTGGATGGCA TCACCAACAC AATGGACATG	9540
GGTTTGGGTG GACTCCAGGA GTTGGTGATG GACAGGGAGG CCTGGCGTGC TACGGAAGCG	9600
GTTTATGGGG TCACAAAGAC TGAGTGACTG AACTGAGCTG AACTGAATGG AAATGAGGTA	9660
TACAGCAAAG TGGGGATTTT TTAGATAATA AGAATATAACA CATAACATAG TGTATACTCA	9720
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ACCGTAGCCT TCCAGGTTTC TTCTGTCCAC AGAATTCTCC AAGGCAAGAA TACTGGAGTG	9840
GGTAGCCATT TCCTCCTCCA GGGGATCCTC CCGACCCAGG GATTGAACCG GCATCTCCTG	9900
TATTGGCAGG TGGATTCTTT ACCACTGTGC CACCAAGGGAA GCGCGTGTAA CTCTCTATGT	9960
CCCACTTAAT TACCAAAGCT GCTCCAAGAA AAAGCCCCTG TGCCCTCTGA GCTTCCCGGC	10020
CTGCAGAGGG TGGTGGGGGT AGACTGTGAC CTGGGAACAC CCTCCCGCTT CAGGACTCCC	10080
GGGCCACGTG ACCCACAGTC CTGCAGACAG CGGGTAGCT CTGCTCTCA AGGCTCATTA	10140
TCTTAAAAAA AAACTGAGGT CTATTTGTG ACTTCGCTGC CGTAACCTCT GAACATCCAG	10200

TGCGATGGAC AGGACCTCCT CCCCAGGCCT CAGGGGCTTC AGGGAGCCAG CCTTCACCTA	10260
TGAGTCACCA GACACTCGGG GGTGGCCCCG CCTTCAGGGT GCTCACAGTC TTCCCATCGT	10320
CCTGATCAAA GAGCAAGACC AATGACTTCT TAGGAGCAAG CAGACACCCA CAGGACACTG	10380
AGGTTCACCA GAGCTGAGCT GTCCTTTGA ACCTAAAGAC ACACAGCTCT CGAAGGTTT	10440
CTCTTTAAC TGGATTTAAG GCCTACTTGC CCCTCAAGAG GGAAGACAGT CCTGCATGTC	10500
CCCAGGACAG CCACTCGGTG GCATCCGAGG CCACTTAGTA TTATCTGACC GCACCCCTGGA	10560
ATTAATCGGT CCAAACCTGGA CAAAAACCTT GGTGGGAAGT TTCATCCCAG AGGCCTCAAC	10620
CATCCTGCTT TGACCACCT GCATCTTTT TTCTTTATG TGTATGCATG TATATATATA	10680
TATATATTTT TTTTTTTTTC ATTTTTGGC TGTGCTGGCT GTTCGTTGCA GTTCGGTGCG	10740
CAGGCTTCTC TCTAGTTCT CTCTAGTCTT CTCTTATCAC AGAGCAGTCT CTAGACGATC	10800
GACGCGT	10807

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 47 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

AATTCCGATC GACGCGTCGA CGATATACTC TAGACGATCG ACGCGTA

47

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 24 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(vii) IMMEDIATE SOURCE:
(B) CLONE: BLGAMP3

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

TGGATCCCCT GCCGGTGCCT CTGG

24

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(vii) IMMEDIATE SOURCE:
(B) CLONE: BLGAMP4

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

AACGCGTCAT CCTCTGTGAG CCAG

24

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(vii) IMMEDIATE SOURCE:
(B) CLONE: ZC6839

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

ACTACGTAGT

10

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 42 base pairs
(B) TYPE: nucleic acid

- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

- (vii) IMMEDIATE SOURCE:
(B) CLONE: ZC6632

- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

CGACGCGGAT CCTACGTACC TGCAGCCATG TTTTCCATGA GG

42

- (2) INFORMATION FOR SEQ ID NO:13:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 21 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

- (vii) IMMEDIATE SOURCE:
(B) CLONE: ZC6627

- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

AGGGCTTCGG CAAGCTTCAG G

21

- (2) INFORMATION FOR SEQ ID NO:14:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 24 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

- (vii) IMMEDIATE SOURCE:
(B) CLONE: ZC6521

- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

GCCAAAGACT TACTTCCCTC TAGA

24

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 30 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(vii) IMMEDIATE SOURCE:

- (B) CLONE: ZC6520

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

GCATGAACGT CGCGTGGTGG TTGTGCTACC

30

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 30 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(vii) IMMEDIATE SOURCE:

- (B) CLONE: ZC6519

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

ACCACCGCAG TTTCATGCTC TAAAACCGTT

30

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 36 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(vii) IMMEDIATE SOURCE:

- (B) CLONE: ZC6518

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

GCTGCGGGAT CCTACGTACT AGGGGGACAG GGAAGG

36

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 45 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(vii) IMMEDIATE SOURCE:

(B) CLONE: ZC6629

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

CGACGCGAAT TCTACGTACC TGCAGCCATG AAAAGGATGG TTTCT

45

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 45 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(vii) IMMEDIATE SOURCE:

(B) CLONE: ZC6630

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

CGACGCGAAT TCTACGTACC TGCAGCCATG AAACATCTAT TATTG

45

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 21 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

82

(vii) IMMEDIATE SOURCE:
(B) CLONE: ZC6625

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

GTGAGATTTT CAGATCTTGT C

21

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(vii) IMMEDIATE SOURCE:
(B) CLONE: ZC6626

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

AAGAATTACT GTGGCCTACC A

21

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 33 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(vii) IMMEDIATE SOURCE:
(B) CLONE: ZC6624

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

GCTGCGGAAT TCTACGTACT ATTGCTGTGG GAA

33

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 45 base pairs
(B) TYPE: nucleic acid

83

- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

- (vii) IMMEDIATE SOURCE:
(B) CLONE: ZC6514

- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

CGACGCGGAT CCTACGTACC TGCAGCCATG AGTTGGTCCT TGCAC

45

- (2) INFORMATION FOR SEQ ID NO:24:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 21 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

- (vii) IMMEDIATE SOURCE:
(B) CLONE: ZC6517

- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

GTCTCTGGTA GCAACATACT A

21

- (2) INFORMATION FOR SEQ ID NO:25:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 22 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

- (vii) IMMEDIATE SOURCE:
(B) CLONE: ZC6516

- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

GGGTTCCTAG CCCTACTAGT AG

22

- (2) INFORMATION FOR SEQ ID NO:26:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 22 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(vii) IMMEDIATE SOURCE:

- (B) CLONE: ZC6515

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

GGGTTTCTAG CCCTACTAGT AG

22

(2) INFORMATION FOR SEQ ID NO:27:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 47 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

AAGCTACGCG TCGATCGTCT AGAGTATATC GTCGACGCGT CGATCGG

Claims

1. A method for producing fibrinogen comprising:
providing a first DNA segment encoding a secretion signal operably linked to a fibrinogen $\text{A}\alpha$ chain, a second DNA segment encoding a secretion signal operably linked to a fibrinogen $\text{B}\beta$ chain, and a third DNA segment encoding a secretion signal operably linked to a fibrinogen γ chain, wherein each of said first, second and third segments is operably linked to additional DNA segments required for its expression in the mammary gland of a host female mammal;

introducing said DNA segments into a fertilized egg of a non-human mammalian species;

inserting said egg into an oviduct or uterus of a female of said species to obtain offspring carrying said DNA constructs;

breeding said offspring to produce female progeny that express said first, second and third DNA segments and produce milk containing biocompetent fibrinogen encoded by said segments;

collecting milk from said female progeny;
and recovering the fibrinogen from the milk.

2. A method according to claim 1 wherein said species is selected from the group consisting of sheep, pigs, goats and cattle.

3. A method according to claim 1 wherein each of said first, second and third DNA segments comprises an intron.

4. A method according to claim 1 wherein the molar ratio of said first, second and third DNA segments is within the range of 0.5-1:0.5-1:0.5-1.

5. A method according to claim 1 wherein each of said first, second and third DNA segments is operably linked to a transcription promoter selected from the group consisting

of casein, β -lactoglobulin, α -lactalbumin and whey acidic protein gene promoters.

6. A method according to claim 1 wherein said first, second and third DNA segments are expressed under the control of a β -lactoglobulin promoter.

7. A method according to claim 1 wherein said introducing step comprises injecting said first, second and third DNA segments into a pronucleus of said fertilized egg.

8. A method according to claim 1 wherein said fibrinogen is human fibrinogen.

9. A method according to claim 1 wherein said second DNA segment comprises a sequence of nucleotides as shown in SEQ ID NO: 3 from nucleotide 470 to nucleotide 8100.

10. A method according to claim 1 wherein said second DNA segment comprises a sequence of nucleotides as shown in SEQ ID NO: 3 from nucleotide 512 to nucleotide 8100.

11. A method of producing fibrinogen comprising:
incorporating a first DNA segment encoding a secretion signal operably linked to an $A\alpha$ chain of fibrinogen into a β -lactoglobulin gene to produce a first gene fusion;

incorporating a second DNA segment encoding a secretion signal operably linked to a $B\beta$ chain of fibrinogen into a β -lactoglobulin gene to produce a second gene fusion;

incorporating a third DNA segment encoding a secretion signal operably linked to a γ chain of fibrinogen into a β -lactoglobulin gene to produce a third gene fusion;

introducing said first, second and third gene fusions into the germ line of a non-human mammal so that said DNA segments are expressed in a mammary gland of said mammal or its female progeny and biocompetent fibrinogen is secreted into milk of said mammal or its female progeny;

obtaining milk from said mammal or its female progeny; and
recovering said fibrinogen from said milk.

12. A method according to claim 11 wherein said mammal is a sheep, pig, goat or bovine.

13. A method according to claim 11 wherein each of said first, second and third gene fusions comprises an intron.

14. A method according to claim 11 wherein the molar ratio of said first, second and third gene fusions introduced is within the range of 0.5-1:0.5-1:0.5-1.

15. A method according to claim 11 wherein said introducing step comprises injecting said first, second and third gene fusions into a pronucleus of a fertilized egg and inserting said egg into an oviduct of a pseudopregnant female to produce female offspring carrying said gene fusions in the germ line.

16. A method for producing fibrinogen comprising:
providing a transgenic female non-human mammal carrying in its germline heterologous DNA segments encoding $\text{A}\alpha$, $\text{B}\beta$ and γ chains of fibrinogen, wherein said segments are expressed in a mammary gland of said mammal and fibrinogen encoded by said segments is secreted into milk of said mammal;
collecting milk from said mammal; and
recovering said fibrinogen from said milk.

17. A method according to claim 16 wherein said mammal is a sheep, pig, goat or bovine.

18. A non-human mammalian embryo containing in its nucleus heterologous DNA segments encoding $\text{A}\alpha$, $\text{B}\beta$ and γ chains of fibrinogen.

19. A transgenic non-human female mammal that produces recoverable amounts of human fibrinogen in its milk.

20. A process for producing a transgenic offspring of a mammal comprising:

providing a first DNA segment encoding a fibrinogen $A\alpha$ chain, a second DNA segment encoding a fibrinogen $B\beta$ chain, and a third DNA segment encoding a fibrinogen γ chain, wherein each of said first, second and third segments is operably linked to additional DNA segments required for its expression in a mammary gland of a host female mammal and secretion into milk of said host female mammal;

introducing said DNA segments into a fertilized egg of a mammal of a non-human species;

inserting said egg into an oviduct or uterus of a female of said non-human species to obtain an offspring carrying said first, second and third DNA segments.

21. A process according to claim 20 wherein said offspring is female.

22. A process according to claim 20 wherein said offspring is male.

23. A non-human mammal produced according to the process of claim 20.

24. A non-human mammal according to claim 23 wherein said mammal is female.

25. A female mammal according to claim 24 that produces milk containing biocompetent fibrinogen encoded by said DNA segments.

26. A non-human mammal according to claim 23 wherein said mammal is male.

27. A non-human mammal carrying in its germline DNA segments encoding heterologous $A\alpha$, $B\beta$ and γ chains of fibrinogen, wherein female progeny of said mammal express said DNA segments in a mammary gland to produce biocompetent fibrinogen.

28. A mammal according to claim 27 wherein said mammal is female.

29. A mammal according to claim 27 wherein said mammal is male.

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FIGURE 1

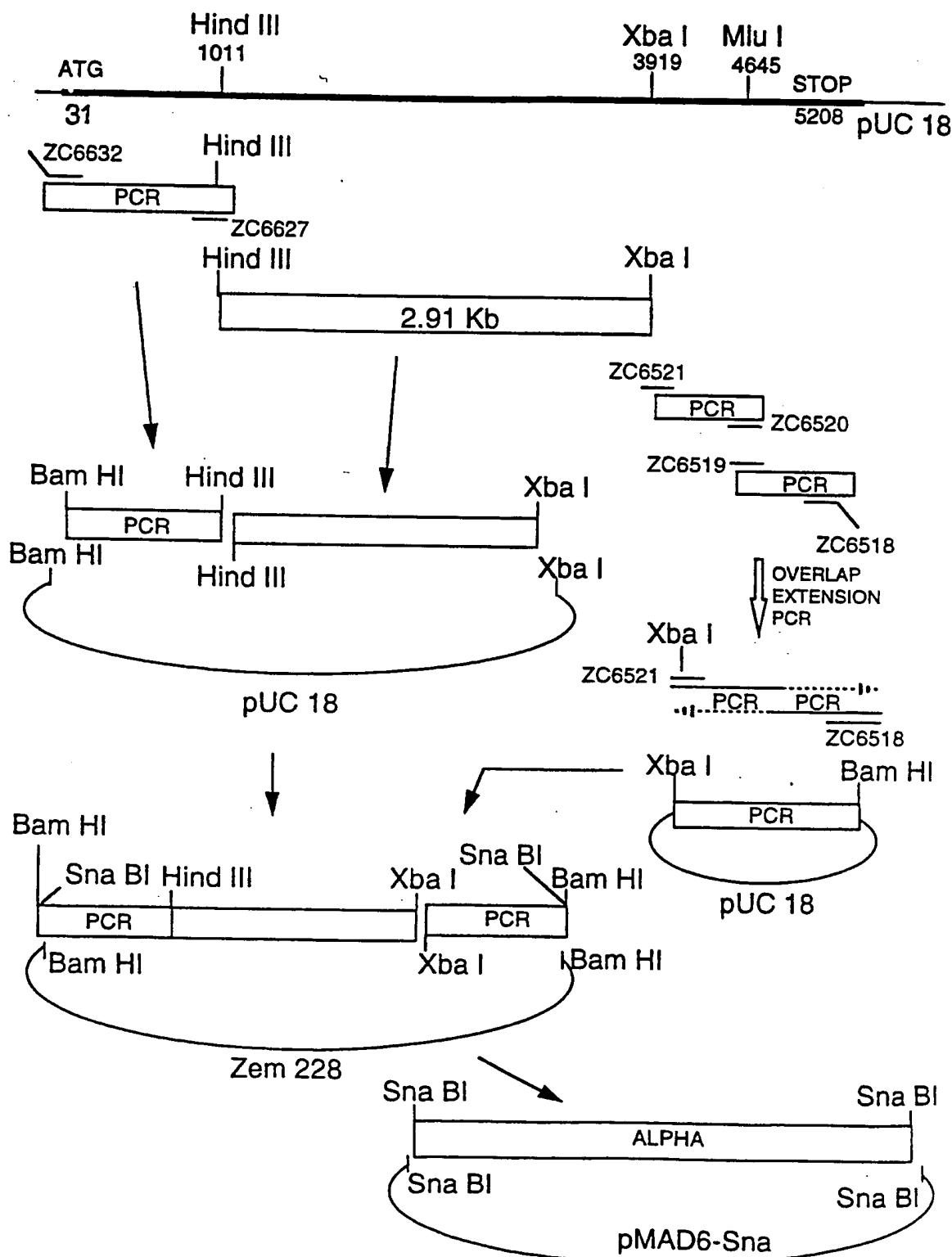


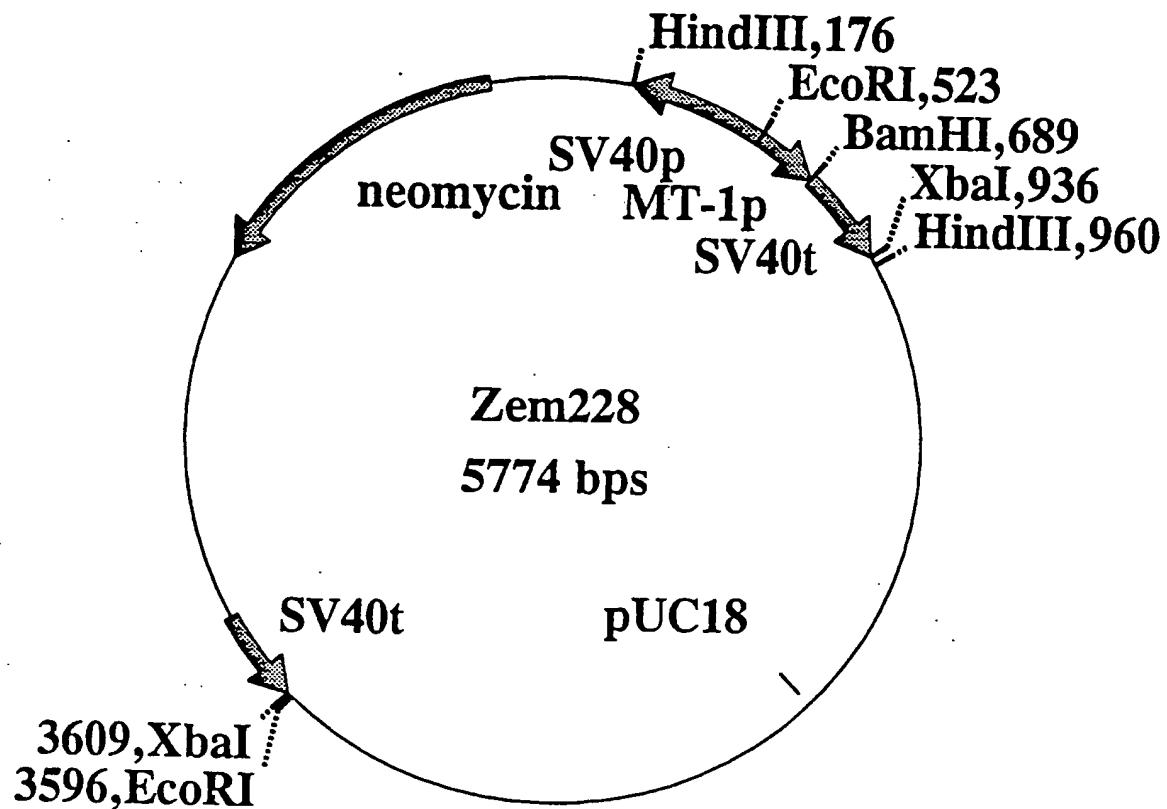
FIGURE 2

FIGURE 3

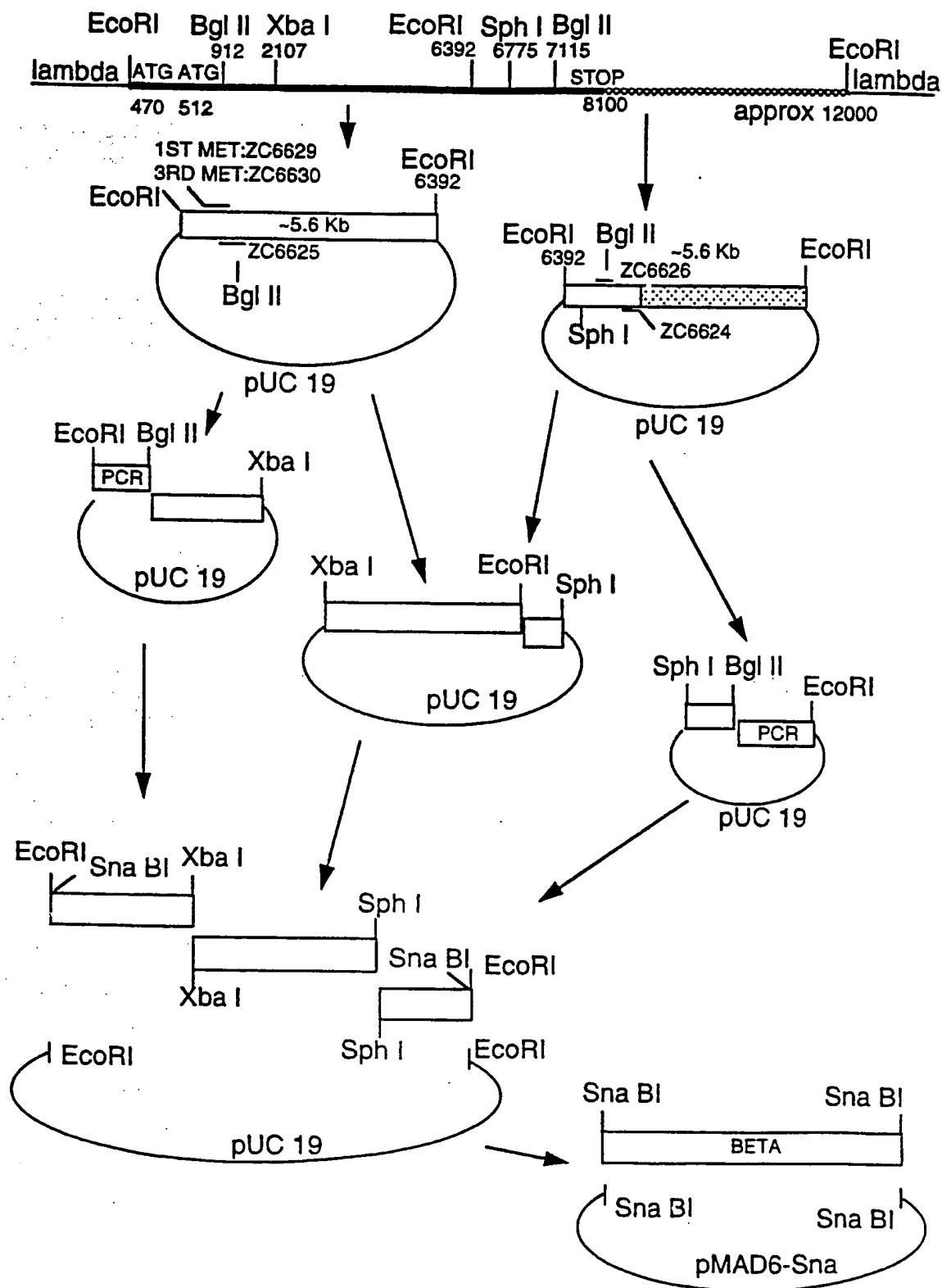


FIGURE 4

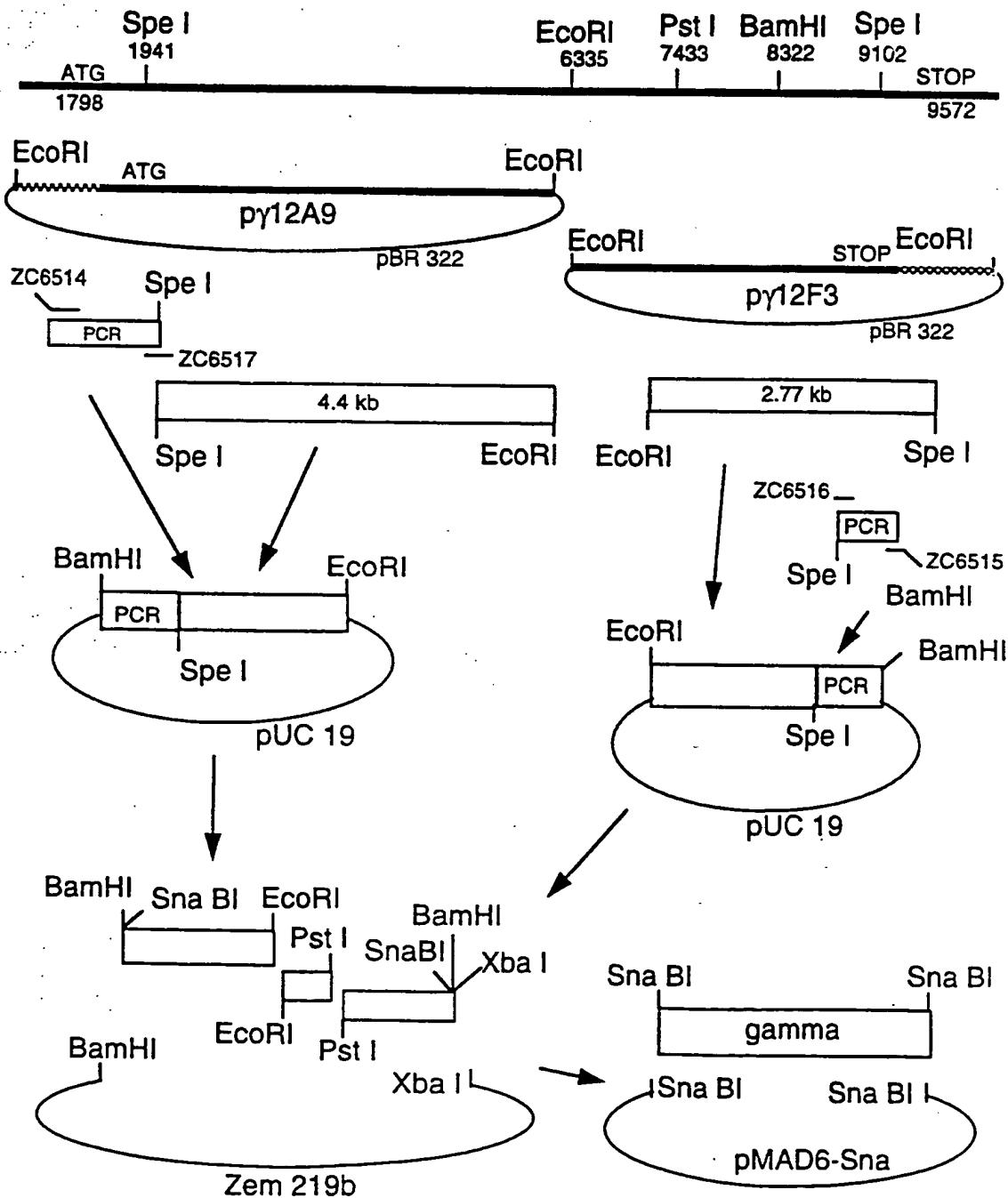
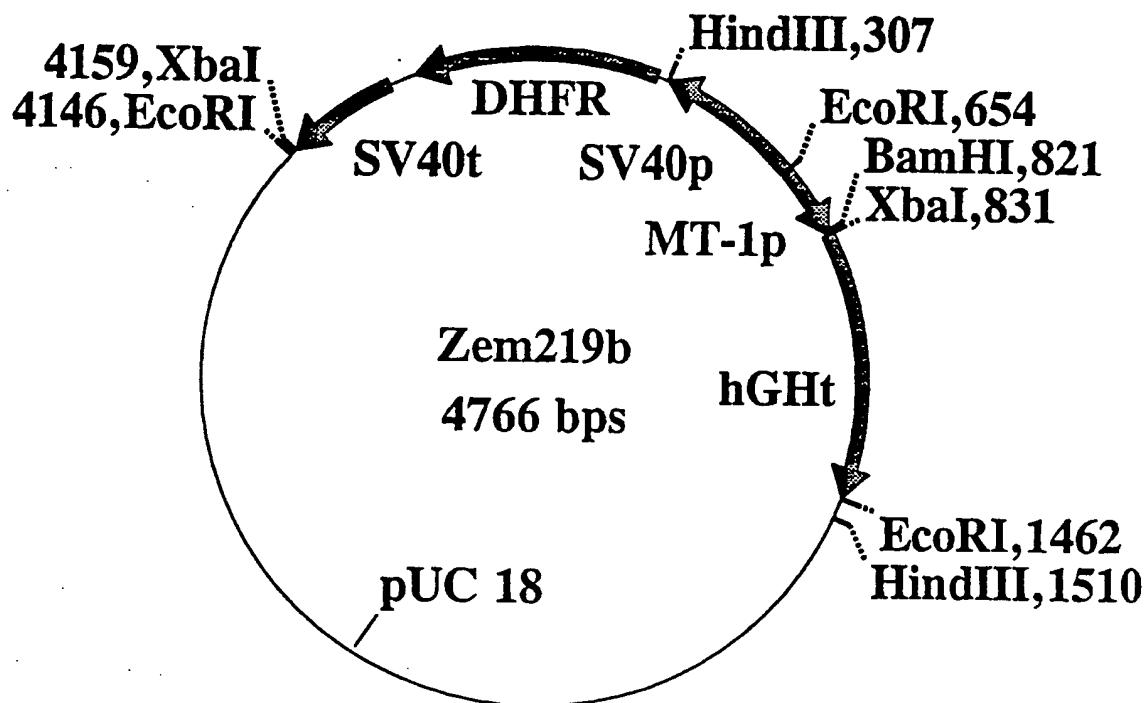


FIGURE 5

INTERNATIONAL SEARCH REPORT

In National Application No
PCT/US 95/02648

A. CLASSIFICATION OF SUBJECT MATTER

IPC 6	C12N15/89	C12N15/90	C12N15/63	C12N15/62	C12N15/85
	A01K67/027	C07K14/75	//C07K14/47		

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6	A01K C07K
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Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

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Y	<p>----</p> <p>----</p>	1-18, 20-26, 29

Further documents are listed in the continuation of box C.

Patent family members are listed in annex.

* Special categories of cited documents :

- *A* document defining the general state of the art which is not considered to be of particular relevance
- *E* earlier document but published on or after the international filing date
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- *O* document referring to an oral disclosure, use, exhibition or other means
- *P* document published prior to the international filing date but later than the priority date claimed

- *T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- *X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
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- *&* document member of the same patent family

Date of the actual completion of the international search

27 June 1995

Date of mailing of the international search report

- 3. 07. 95

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Gac, G

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 95/02648

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

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Y	J. CONTROL. RELEASE, vol. 29, 1994 pages 213-221, SANG HE LEE ET AL. 'Production of biomedical proteins in the milk of transgenic dairy cows : the state of the art' proc. fourth int. symp. dispos. delivery peptide drugs, Leiden, Netherlands, 23-25 April 1993 see page 215 see page 218 ----	1-29
Y	WO,A,92 11358 (THE AGRICULTURAL AND FOOD RESEARCH COUNCIL) 9 July 1992 see the whole document ----	1-29
A	WO,A,88 00239 (PHARMACEUTICAL PROTEINS LTD) 14 January 1988 cited in the application see the whole document ----	1-29
A	WO,A,90 05188 (PHARMACEUTICAL PROTEINS LIMITED) 17 May 1990 see pages 6-10,12,35-46,51-53 and claims ----	1-10, 15-17
A	WO,A,91 08216 (GENPHARM INTERNATIONAL) 13 June 1991 see the whole document ----	1-29
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International Application No

PCT/US 95/02648

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